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(54) Title: DSP-16 DUAL-SPECIFICITY PHOSPHATASE

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          1.......10........20........30........40........50........60........70........80
 PYST1  DGSPLSNSQPSFPVEILPFLYLGCAKDSTNLDVLEEFGIKYILNVTP-------NLPNLFENAGEFKYKQIPISDHWSQN
 MKP-7  DGSPVPSSQPAFPVQILPYLYLGCAKDSTNLDVLGKYGIKYILNVTP-------NLPNAFEHGGEFTYKQIPISDHWSQN
 MKP-4  ATPPPVGLRASFPVQILPNLYLGSARDSANLESLAKLGIRYILNVTP-------NLPNFFEKNGDFHYKQIPISDHWSQN
 hVH5   LSQPCLPVPSVGLTRILPHLYLGSQKDVLNKDLMTQNGISYVLNASN-------SCPKP-DFICESRFMRVPIHDNYCEK
 DSP-16 ISQPCLPVANIGPTRILPNLYLGCQRDVLNKELMQQNGIGYVLNASN-------TCPKP-DFIPESHFLRVPVNDSFCEK
 PAC1   SDPRVPIYDQGGPVEILPYLYLGSCNHSSDLQGLQACGITAVLNVSA-------SCPNHFE--GLFHYKSIPVEDNQMVE
 MKP-1  SSCSTPLYDQGGPVEILPFLYLGSAYHASRKDMLDALGITALINVSA-------NCPNHFE--GHYQYKSIPVEDNHKAD
 MKP-2  SSCGTPLHDQGGPVEILPFLYLGSAYHAARRDMLDALGITALLNVSS-------DCPNHFE--GHYQYKCIPVEDNHKAD
 MKP-5  NVSYRPAYDQGGPVEILPFLYLGSAYHASKCEFLANLHITALLNVSR-------RTSEACM--THLHYKWIPVEDSHTAD
 VHR    DGSGCYSLPSQPCNEVTPRIYVGNASVAQDIPKLQKLGITHVLNAAEGRSFMHVNTNANFYKDSGITYLGIKANDTQEFN
Consensus  ..........gpve!lP.lYlGsa........$..lglt..LNvs.........n.pn.fe..g...!k.!p..D.....


          81......90.......100.......110.......120.......130.......140.......150.......160.164
 PYST1  LSQFFPEAISFIDEA-RGKNCGVLVHCLAGISRSVTVTVAYLMQKLNLSMHDAYDIVKMKKSNISPNFNFMGQLLDFERTLGLS
 MKP-7  LSQFFPEAISFIDEA-RSKKCGVLVHCLAGISRSVTVTVAYLMQKMNLSLNDAYDFVKRKKSNISPNFNFMGQLLDFERTLGLS
 MKP-4  LSRFFPEAIEFIDEA-LSQNCGVLVHCLAGVSRSVTVTVAYLMQKLHLSLNDAYDLVKRKKSNISPNFNFMGQLLDFERSLRLE
 hVH5   LLPWLDKSIEFIDKA-KLSSCQVIVHCLAGISRSATIAIAYIMKTMGMSSDDAYRFVKDRRPSISPNFNFLGQLLEYERTLKLL
 DSP-16 ILPWLDKSVDFIEKA-KASNGCVLVHCLAGISRSATIAIAYIMKRMDMSLDEAYRFVKEKRPTISPNFNFLGQLLDYEKKIKNQ
 PAC1   ISAWFQEAISFIDSV-KNSGGRVLVHCQAGISRSATICLAYLIQSHRVRLDEAFDFVKQRRGVISPNFSFMGQLLQLETQVLCH
 MKP-1  ISSWFNEAIDFIDSI-KNAGGRVFVHCQAGISRSATICLAYLMRTNRVKLDEAFEFVKQRRSIISPNFSFMGQLLQFESQVLAP
 MKP-2  ISSWFMEAIEYIDAV-KDCRGRVLVHCQAGISRSATICLAYLMMKKRVRLEEAFEFVKQRRSIISPNFSFMGQLLQFESQVLAT
 MKP-5  ISSHFQEAIDFIDCV-REKGGKVLVHCEAGISRSPTICMAYLMKTKQFRLKEAFDYIKQRRSMVSPNFGFMGQLLQYESEILPS
 VHR    LSAYFERAADFIDQALAQKNGRVLVHCREGYSRSPTLVIAYLMNRQKMDVKSALSIVRQNRE-IGPNDGFLAQLCQLNDRLAKE
Consensus  ls..f.eai.FI#.a.....g.VlVHC.aGiSRS.Ti..AYlM......l..A..f!kq.rs.!sPNf.F$gQLl#.#..l...
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(57) Abstract: Compositions and methods are provided for the treatment of conditions associated with cell proliferation, cell differentiation and cell survival. In particular, the dual-specificity phosphatase DSP-16, and polypeptide variants thereof that stimulate dephosphorylation of DSP-16 substrates, are provided. The polypeptides may be used, for example, to identify antibodies and other agents that inhibit DSP-16 activity. The polypeptides and agents may be used to modulate cell proliferation, differentiation and survival.

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DSP-16 DUAL-SPECIFICITY PHOSPHATASE

TECHNICAL FIELD

The present invention relates generally to compositions and methods useful for treating conditions associated with defects in cell proliferation, cell

5   differentiation and/or cell survival. The invention is more particularly related to dual-specificity protein phosphatases, and polypeptide variants thereof. The present invention is also related to the use of such polypeptides to identify antibodies and other agents, including small molecules, that modulate signal transduction leading to proliferative responses, cell differentiation and/or cell survival.

10 · BACKGROUND OF THE INVENTION

Mitogen-activated protein kinases (MAP-kinases) are present as components of conserved cellular signal transduction pathways that have a variety of conserved members. MAP-kinases are activated by phosphorylation at a dual phosphorylation motif with the sequence Thr-X-Tyr (by MAP-kinase kinases), in which

15   phosphorylation at the tyrosine and threonine residues is required for activity. Activated MAP-kinases phosphorylate several transduction targets, including transcription factors. Inactivation of MAP-kinases is mediated by dephosphorylation at this site by dual-specificity phosphatases referred to as MAP-kinase phosphatases. In higher eukaryotes, the physiological role of MAP-kinase signaling has been correlated with cellular events

20   such as proliferation, oncogenesis, development and differentiation. Accordingly, the ability to regulate signal transduction via these pathways could lead to the development of treatments and preventive therapies for human diseases associated with MAP-kinase signaling, such as cancer.

Dual-specificity protein tyrosine phosphatases (dual-specificity

25   phosphatases) are phosphatases that dephosphorylate both phosphotyrosine and phosphothreonine/serine residues (Walton et al., *Ann. Rev. Biochem.* 62:101-120, 1993). Several dual-specificity phosphatases that inactivate a MAP-kinase have been identified, including MKP-1 (WO 97/00315; Keyse and Emslie, *Nature 59*:644-647, 1992), MKP-2 (WO97/00315), MKP-4, MKP-5, MKP-7, Hb5 (WO 97/06245), PAC1

(Ward et al., *Nature 367*:651-654, 1994), HVH2 (Guan and Butch, *J. Biol. Chem. 270*:7197-7203, 1995) and PYST1 (Groom et al., *EMBO J. 15*:3621-3632, 1996). Expression of certain dual-specificity phosphatases is induced by stress or mitogens, but others appear to be expressed constitutively in specific cell types. The regulation of

5 dual-specificity phosphatase expression and activity is critical for control of MAP-kinase mediated cellular functions, including cell proliferation, cell differentiation and cell survival. For example, dual-specificity phosphatases may function as negative regulators of cell proliferation. It is likely that there are many such dual-specificity phosphatases, with varying specificity with regard to cell type or activation. However,

10 the regulation of dual specificity phosphatases remains poorly understood and only a relatively small number of dual-specificity phosphatases have been identified.

Accordingly, there is a need in the art for an improved understanding of MAP-kinase signaling, and the regulation of dual-specificity phosphatases within MAP-kinase signaling cascades. An increased understanding of dual-specificity phosphatase

15 regulation may facilitate the development of methods for modulating the activity of proteins involved in MAP-kinase cascades, and for treating conditions associated with such cascades. The present invention fulfills these needs and further provides other related advantages.

SUMMARY OF THE INVENTION

20   Briefly stated, the present invention provides compositions and methods for identifying agents capable of modulating cellular proliferative responses. In one aspect, the present invention provides isolated DSP-16 polypeptides having the sequence of DSP-16 recited in SEQ ID NO:2, or a variant thereof that differs in one or more amino acid deletions, additions, insertions or substitutions at no more than 50% of

25 the residues in SEQ ID NO:2, such that the polypeptide retains the ability to dephosphorylate an activated MAP-kinase.

Within further aspects, the present invention provides an isolated polynucleotide that encodes at least ten consecutive amino acids of a polypeptide having a sequence corresponding to SEQ ID NO:2. In certain embodiments the invention

30 provides an isolated polynucleotide that encodes at least fifteen consecutive amino acids

of a polypeptide having a sequence corresponding to SEQ ID NO:2. Certain such polynucleotides encode a DSP-16 polypeptide. Still further, polynucleotides may be antisense polynucleotides that comprise at least 15 consecutive nucleotides complementary to a portion of a DSP-16 polynucleotide and/or that detectably hybridize

5   to the complement of the sequence recited in SEQ ID NO:1 under conditions that include a wash in 0.1X SSC and 0.1% SDS at 50°C for 15 minutes. Also provided are expression vectors comprising any of the foregoing polynucleotides, and host cells transformed or transfected with such expression vectors.

The present invention further provides, within other aspects, methods for

10   producing a DSP-16 polypeptide, comprising the steps of: (a) culturing a host cell as described above under conditions that permit expression of the DSP-16 polypeptide; and (b) isolating DSP-16 polypeptide from the host cell culture.

Also provided by the present invention are isolated antibodies, and antigen binding fragments thereof, that specifically bind to a DSP-16 polypeptide such

15   as a polypeptide having the sequence of SEQ ID NO:2.

The present invention further provides, within other aspects, pharmaceutical compositions comprising a polypeptide, polynucleotide, antibody or fragment thereof as described above in combination with a physiologically acceptable carrier.

20   Within further aspects, the present invention provides methods for detecting DSP-16 expression in a sample, comprising: (a) contacting a sample with an antibody or an antigen-binding fragment thereof as described above, under conditions and for a time sufficient to allow formation of an antibody/DSP-16 complex; and (b) detecting the level of antibody/DSP-16 complex.

25   Within still other aspects, the present invention provides methods for detecting DSP-16 expression in a sample, comprising: (a) contacting a sample with an antisense polynucleotide as described above; and (b) detecting in the sample an amount of DSP-16 polynucleotide that hybridizes to the antisense polynucleotide. The amount of DSP-16 polynucleotide that hybridizes to the antisense polynucleotide may be

30   determined, for example, using polymerase chain reaction or a hybridization assay.

The invention also provides DSP-16 polypeptides useful in screening assays for modulators of enzyme activity and/or substrate binding. Methods are also provided, within other aspects, for screening for an agent that modulates DSP-16 activity, comprising the steps of: (a) contacting a candidate agent with a DSP-16 5 polypeptide as described above, under conditions and for a time sufficient to permit interaction between the polypeptide and candidate agent; and (b) subsequently evaluating the ability of the polypeptide to dephosphorylate a DSP-16 substrate, relative to a predetermined ability of the polypeptide to dephosphorylate the DSP-16 substrate in the absence of candidate agent. Such methods may be performed *in vitro* or in a cellular 10 environment (*e.g.*, within an intact cell).

Within further aspects, methods are provided for screening for an agent that modulates DSP-16 activity, comprising the steps of: (a) contacting a candidate agent with a cell comprising a DSP-16 promoter operably linked to a polynucleotide encoding a detectable transcript or protein, under conditions and for a time sufficient to 15 permit interaction between the promoter and candidate agent; and (b) subsequently evaluating the expression of the polynucleotide, relative to a predetermined level of expression in the absence of candidate agent.

Also provided are methods for modulating a proliferative response in a cell, comprising contacting a cell with an agent that modulates DSP-16 activity.

20 Within further aspects, methods are provided for modulating differentiation of a cell, comprising contacting a cell with an agent that modulates DSP-16 activity.

The present invention further provides methods for modulating cell survival, comprising contacting a cell with an agent that modulates DSP-16 activity.

25 Within related aspects, the present invention provides methods for treating a patient afflicted with a disorder associated with DSP-16 activity (or treatable by administration of DSP-16), comprising administering to a patient a therapeutically effective amount of an agent that modulates DSP-16 activity. Such disorders include Duchenne Muscular Dystrophy, as well as cancer, graft-versus-host disease, 30 autoimmune diseases, allergies, metabolic diseases, abnormal cell growth, abnormal cell proliferation and cell cycle abnormalities.

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Within further aspects, DSP-16 substrate trapping mutant polypeptides are provided. Such polypeptides differ from the sequence recited in SEQ ID NO:2 in one or more amino acid deletions, additions, insertions or substitutions at no more than 50% of the residues in SEQ ID NO:2, such that the polypeptide binds to a substrate with

5 an affinity that is not substantially diminished relative to DSP-16, and such that the ability of the polypeptide to dephosphorylate a substrate is reduced relative to DSP-16. Within certain specific embodiments, a substrate trapping mutant polypeptide contains a substitution at position 213 or position 244 of SEQ ID NO:2.

The present invention further provides, within other aspects, methods for

10 screening a molecule for the ability to interact with DSP-16, comprising the steps of: (a) contacting a candidate molecule with a polypeptide as described above under conditions and for a time sufficient to permit the candidate molecule and polypeptide to interact; and (b) detecting the presence or absence of binding of the candidate molecule to the polypeptide. The step of detecting may comprise, for example, an affinity

15 purification step, a yeast two hybrid screen or a screen of a phage display library.

In one aspect, the present invention provides isolated DSP-16 polypeptides comprising the sequence of DSP-16 alternate form recited in SEQ ID NO:21, or a variant thereof that differs in one or more amino acid deletions, additions, insertions or substitutions at no more than 50% of the residues in SEQ ID NO:21, such

20 that the polypeptide retains the ability to dephosphorylate an activated MAP-kinase.

Within further aspects, the present invention provides an isolated polynucleotide that encodes at least ten consecutive amino acids of a polypeptide having a sequence corresponding to SEQ ID NO:21. In certain embodiments the invention provides an isolated polynucleotide that encodes at least fifteen consecutive amino acids

25 of a polypeptide having a sequence corresponding to SEQ ID NO:21. Certain such polynucleotides encode a DSP-16 alternate form polypeptide. Still further, polynucleotides may be antisense polynucleotides that comprise at least 15 consecutive nucleotides complementary to a portion of a DSP-16 alternate form polynucleotide and/or that detectably hybridize to the complement of the sequence recited in SEQ ID

30 NO:20 under conditions that include a wash in 0.1X SSC and 0.1% SDS at 60°C for 15

minutes. Also provided are expression vectors comprising any of the foregoing polynucleotides, and host cells transformed or transfected with such expression vectors.

The present invention further provides, within other aspects, methods for producing a DSP-16 alternate form polypeptide, comprising the steps of: (a) culturing a host cell as described above under conditions that permit expression of the DSP-16 alternate form polypeptide; and (b) isolating DSP-16 alternate form polypeptide from the host cell culture.

Also provided by the present invention are isolated antibodies, and antigen binding fragments thereof, that specifically bind to a DSP-16 alternate form polypeptide such as a polypeptide having the sequence of SEQ ID NO:21.

The present invention further provides, within other aspects, pharmaceutical compositions comprising a polypeptide, polynucleotide, antibody or fragment thereof as described above in combination with a physiologically acceptable carrier.

Within further aspects, the present invention provides methods for detecting DSP-16 alternate form expression in a sample, comprising: (a) contacting a sample with an antibody or an antigen-binding fragment thereof as described above, under conditions and for a time sufficient to allow formation of an antibody/DSP-16 alternate form complex; and (b) detecting the level of antibody/DSP-16 alternate form complex.

Within still other aspects, the present invention provides methods for detecting DSP-16 alternate form expression in a sample, comprising: (a) contacting a sample with an antisense polynucleotide as described above; and (b) detecting in the sample an amount of DSP-16 alternate form polynucleotide that hybridizes to the antisense polynucleotide. The amount of DSP-16 alternate form polynucleotide that hybridizes to the antisense polynucleotide may be determined, for example, using polymerase chain reaction or a hybridization assay.

The invention also provides DSP-16 alternate form polypeptides useful in screening assays for modulators of enzyme activity and/or substrate binding. Methods are also provided, within other aspects, for screening for an agent that modulates DSP-16 alternate form activity, comprising the steps of: (a) contacting a

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candidate agent with a polypeptide as described above, under conditions and for a time sufficient to permit interaction between the polypeptide and candidate agent; and (b) subsequently evaluating the ability of the polypeptide to dephosphorylate a DSP-16 alternate form substrate, relative to a predetermined ability of the polypeptide to

5   dephosphorylate the DSP-16 alternate form substrate in the absence of candidate agent. Such methods may be performed *in vitro* or in a cellular environment (*e.g.*, within an intact cell).

Within further aspects, methods are provided for screening for an agent that modulates DSP-16 alternate form activity, comprising the steps of: (a) contacting a

10   candidate agent with a cell comprising a DSP-16 alternate form promoter operably linked to a polynucleotide encoding a detectable transcript or protein, under conditions and for a time sufficient to permit interaction between the promoter and candidate agent; and (b) subsequently evaluating the expression of the polynucleotide, relative to a predetermined level of expression in the absence of candidate agent.

15   Also provided are methods for modulating a proliferative response in a cell, comprising contacting a cell with an agent that modulates DSP-16 alternate form activity.

Within further aspects, methods are provided for modulating differentiation of a cell, comprising contacting a cell with an agent that modulates DSP-

20   16 alternate form activity.

The present invention further provides methods for modulating cell survival, comprising contacting a cell with an agent that modulates DSP-16 alternate form activity.

Within related aspects, the present invention provides methods for

25   treating a patient afflicted with a disorder associated with DSP-16 alternate form activity (or treatable by administration of DSP-16 alternate form), comprising administering to a patient a therapeutically effective amount of an agent that modulates DSP-16 alternate form activity. Such disorders include cancer, graft-versus-host disease, autoimmune diseases, allergies, metabolic diseases, abnormal cell growth,

30   abnormal cell proliferation and cell cycle abnormalities.

Within further aspects, DSP-16 alternate form substrate trapping mutant polypeptides are provided. Such polypeptides differ from the sequence recited in SEQ ID NO:21 in one or more amino acid deletions, additions, insertions or substitutions at no more than 50% of the residues in SEQ ID NO:21, such that the polypeptide binds to

5   a substrate with an affinity that is not substantially diminished relative to DSP-16 alternate form, and such that the ability of the polypeptide to dephosphorylate a substrate is reduced relative to DSP-16 alternate form. Within certain specific embodiments, a substrate trapping mutant polypeptide contains a substitution at position 213 or position 244 of SEQ ID NO:21.

10   The present invention further provides, within other aspects, methods for screening a molecule for the ability to interact with DSP-16 alternate form, comprising the steps of: (a) contacting a candidate molecule with a DSP-16 alternate form polypeptide or variant thereof as described above under conditions and for a time sufficient to permit the candidate molecule and polypeptide to interact; and (b) detecting

15   the presence or absence of binding of the candidate molecule to the polypeptide. The step of detecting may comprise, for example, an affinity purification step, a yeast two hybrid screen or a screen of a phage display library.

These and other aspects of the present invention will become apparent

20   upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 presents a cDNA sequence for DSP-16 (SEQ ID NO:1), with

25   the start and stop codons shown in bold.

Figure 2 presents the predicted amino acid sequence of DSP-16 (SEQ ID NO:2).

Figure 3 shows a cDNA sequence for a DSP-16 alternate form (SEQ ID NO:20), with the start and stop codons shown in bold.

Figure 4 presents the predicted amino acid sequence of the DSP-16 alternate form (SEQ ID NO:21) encoded by the protein coding region of SEQ ID NO:20.

Figure 5 shows a sequence alignment of DSP-16 with other MAP kinase phosphatases (SEQ ID NOS: 3-12).

DETAILED DESCRIPTION OF THE INVENTION

As noted above, the present invention is generally directed to compositions and methods for modulating (*i.e.*, stimulating or inhibiting) cellular proliferative responses, *in vitro* and *in vivo*. In particular, the present invention provides a dual-specificity phosphatase DSP-16 or DSP-16 alternate form (Figures 1-4; SEQ ID NOs:1, 2, 20, 21), as well as variants thereof and antibodies that specifically bind DSP-16 or DSP-16 alternate form. Also provided herein are methods for using such compounds for screens, detection assays and related therapeutic uses.

DSP-16 POLYPEPTIDES AND POLYNUCLEOTIDES

As used herein, the term "DSP-16 polypeptide" or "DSP-16 alternate form polypeptide" refers to a polypeptide that comprises a DSP-16 sequence as provided herein or a variant of such a sequence. Such polypeptides are capable of dephosphorylating both tyrosine and threonine/serine residues in a DSP-16 substrate, with an activity that is not substantially diminished relative to that of a full length native DSP-16. DSP-16 substrates include activated (*i.e.*, phosphorylated) MAP-kinases. Other substrates may be identified using substrate trapping mutants, as described herein, and include polypeptides having one or more phosphorylated tyrosine, threonine and /or serine residues.

DSP-16 or DSP-16 alternate form polypeptide variants within the scope of the present invention may contain one or more substitutions, deletions, additions and/or insertions. For certain DSP-16 or DSP-16 alternate form variants, the ability of the variant to dephosphorylate tyrosine and threonine residues within a DSP-16 substrate is not substantially diminished. The ability of such a DSP-16 variant to dephosphorylate tyrosine and threonine residues within a DSP-16 substrate may be

enhanced or unchanged, relative to a native DSP-16 or DSP-16 alternate form, or may

be diminished by less than 50%, and preferably less than 20%, relative to native DSP-16

or DSP-16 alternate form. Such variants may be identified using the representative

assays provided herein.

5   Also contemplated by the present invention are modified forms of DSP-

16 and/or DSP-16 alternate form in which a specific function is disabled. For example,

such proteins may be constitutively active or inactive, or may display altered binding or

catalytic properties. Such altered proteins may be generated using well known

techniques, and the altered function confirmed using screens such as those provided

10 herein. Certain modified DSP-16 or DSP-16 alternate form polypeptides are known as

"substrate trapping mutants." Such polypeptides retain the ability to bind a substrate

($i.e.$, K_m is not substantially diminished), but display a reduced ability to

dephosphorylate a substrate ($i.e.$, k_{cat} is reduced, preferably to less than 1 per minute).

Further, the stability of the substrate trapping mutant/substrate complex should not be

15 substantially diminished, relative to the stability of a DSP-16/substrate complex,

including a DSP-5 alternate form/substrate complex. Complex stability may be

assessed based on the association constant (K_a). Determination of K_m, k_{cat} and K_a may

be readily accomplished using standard techniques known in the art (see, e.g., WO

98/04712; Lehninger, Biochemistry, 1975 Worth Publishers, NY) and assays provided

20 herein. Substrate trapping mutants may be generated, for example, by modifying DSP-

16 with an amino acid substitution at position 213 or position 244 (e.g., by replacing the

amino acid aspartate at position 213 with an alanine residue, or by replacing the cysteine

at residue 244 with a serine). Substrate trapping mutants may be used, for example, to

identify DSP-16 substrates. Briefly, the modified DSP-16 or DSP-16 alternate form

25 may be contacted with a candidate substrate (alone or within a mixture of proteins, such

as a cell extract) to permit the formation of a substrate/DSP-16 complex. The complex

may then be isolated by conventional techniques to permit the isolation and

characterization of substrate. The preparation and use of substrate trapping mutants is

described, for example, within PCT Publication No. WO 98/04712.

30   Preferably, a variant contains conservative substitutions. A

"conservative substitution" is one in which an amino acid is substituted for another

amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. Amino acid substitutions may generally be made on the basis of similarity in polarity, charge, solubility, hydrophobicity,

5   hydrophilicity and/or the amphipathic nature of the residues. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine and valine; glycine and alanine; asparagine and glutamine; and serine, threonine, phenylalanine and tyrosine.

10   Other groups of amino acids that may represent conservative changes include: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his. A variant may also, or alternatively, contain nonconservative changes.

In general, modifications may be more readily made in non-critical

15   regions, which are regions of the native sequence that do not substantially change the activity of DSP-16 or DSP-16 alternate form. Non-critical regions may be identified by modifying the DSP-16 sequence in a particular region and assaying the ability of the resulting variant in a phosphatase assay, as described herein. Preferred sequence modifications are made so as to retain the active site domain (VHCLAGISRS, SEQ ID

20   NO:16). Within certain preferred embodiments, such modifications affect interactions between DSP-16 (or DSP-16 alternate form) and cellular components other than DSP-16 substrates. However, substitutions may also be made in critical regions of the native protein, provided that the resulting variant substantially retains the ability to stimulate substrate dephosphorylation. Within certain embodiments, a variant contains

25   substitutions, deletions, additions and/or insertions at no more than 50%, preferably no more than 25%, of the amino acid residues.

Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the activity of the polypeptide. In particular, variants may contain additional amino acid sequences at the

30   amino and/or carboxy termini. Such sequences may be used, for example, to facilitate purification or detection of the polypeptide.

DSP-16 (or DSP-16 alternate form) polypeptides may be prepared using any of a variety of well known techniques. Recombinant polypeptides encoded by DNA sequences as described below may be readily prepared from the DNA sequences using any of a variety of expression vectors known to those having ordinary skill in the art.

5   Expression may be achieved in any appropriate host cell that has been transformed or transfected with an expression vector containing a DNA molecule that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast and higher eukaryotic cells (including mammalian cells), and forms that differ in glycosylation may be generated by varying the host cell or post-isolation processing. Supernatants from

10   suitable host/vector systems which secrete recombinant protein or polypeptide into culture media may be first concentrated using a commercially available filter. Following concentration, the concentrate may be applied to a suitable purification matrix such as an affinity matrix or an ion exchange resin. Finally, one or more reverse phase HPLC steps can be employed to further purify a recombinant polypeptide.

15   Portions and other variants having fewer than about 100 amino acids, and generally fewer than about 50 amino acids, may also be generated by synthetic procedures, using techniques well known to those having ordinary skill in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where

20   amino acids are sequentially added to a growing amino acid chain. *See* Merrifield, *J. Am. Chem. Soc. 85*:2149-2146, 1963. Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Perkin-Elmer, Inc., Applied BioSystems Division (Foster City, CA), and may be operated according to the manufacturer's instructions.

25   A "DSP-16 polynucleotide" is any polynucleotide that encodes at least a portion of a DSP-16 or DSP-16 alternate form polypeptide or a variant thereof, or that is complementary to such a polynucleotide. Preferred polynucleotides comprise at least 15 consecutive nucleotides, preferably at least 30 consecutive nucleotides, that encode a DSP-16 or DSP-16 alternate form polypeptide or that are complementary to such a

30   sequence. Certain polynucleotides encode a DSP-16 or DSP-16 alternate form polypeptide; others may find use as probes, primers or antisense oligonucleotides, as

described below. Polynucleotides may be single-stranded (coding or antisens
double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA mole
Additional coding or non-coding sequences may, but need not, be present wit
polynucleotide of the present invention, and a polynucleotide may, but need n

5   linked to other molecules and/or support materials.

DSP-16 polynucleotides may comprise a native sequence (i.e
endogenous DSP-16 or DSP-16 alternate form sequence, or a portion or splice v
thereof) or may comprise a variant of such a sequence. Polynucleotide variant
contain one or more substitutions, additions, deletions and/or insertions such th;

10 activity of the encoded polypeptide is not substantially diminished, as described a
The effect on the activity of the encoded polypeptide may generally be assess
described herein. Variants preferably exhibit at least about 70% identity, :
preferably at least about 80% identity and most preferably at least about 90% ideni
a polynucleotide sequence that encodes a native DSP-16 or DSP-16 alternate form

15 portion thereof. The percent identity may be readily determined by comp
sequences using computer algorithms well known to those having ordinary skill :
art, such as Align or the BLAST algorithm (Altschul, *J. Mol. Biol. 219*:555-565, ;
Henikoff and Henikoff, *Proc. Natl. Acad. Sci. USA 89*:10915-10919, 1992), wh:
available at the NCBI website (http://www/ncbi.nlm.nih.gov/cgi-bin/BLAST). Dt

20 parameters may be used. Certain variants are substantially homologous to a ꜱ
gene. Such polynucleotide variants are capable of hybridizing under mode'
stringent conditions to a naturally occurring DNA or RNA sequence encoding a ꜱ
DSP-16 or DSP-16 alternate form (or a complementary sequence). Suitable mode'
stringent conditions include, for example, prewashing in a solution of 5 X SSC,>

25 SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-70°C, 5 X SSC, for 1-16 hours
overnight); followed by washing once or twice at 22-65°C for 20-40 minutes wit
or more each of 2X, 0.5X and 0.2X SSC containing 0.05-0.1% SDS. For addil
stringency, conditions may include a wash in 0.1X SSC and 0.1% SDS at 50-60 ꜱ
15-40 minutes. As known to those having ordinary skill in the art, variatiα

30 stringency of hybridization conditions may be achieved by altering the ,
temperature and/or concentration of the solutions used for prehybridiz,

hybridization and wash steps, and suitable conditions may also depend in part on the particular nucleotide sequences of the probe used, and of the blotted, proband nucleic acid sample. Accordingly, it will be appreciated that suitably stringent conditions can be readily selected without undue experimentation where a desired selectivity of the

5   probe is identified, based on its ability to hybridize to one or more certain proband sequences while not hybridizing to certain other proband sequences.

It will also be appreciated by those having ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that encode a polypeptide as described herein. Some of these polynucleotides bear minimal

10   homology to the nucleotide sequence of any native gene. Nonetheless, polynucleotides that vary due to differences in codon usage are specifically contemplated by the present invention.

Polynucleotides may be prepared using any of a variety of techniques. For example, a polynucleotide may be amplified from cDNA prepared from a suitable

15   cell or tissue type, such as human skeletal muscle cells. Such polynucleotides may be amplified via polymerase chain reaction (PCR). For this approach, sequence-specific primers may be designed based on the sequences provided herein, and may be purchased or synthesized.

An amplified portion may be used to isolate a full length gene from a

20   suitable library (*e.g.*, human brain, testis and skeletal muscle cell cDNA) using well known techniques. Within such techniques, a library (cDNA or genomic) is screened using one or more polynucleotide probes or primers suitable for amplification. Preferably, a library is size-selected to include larger molecules. Random primed libraries may also be preferred for identifying 5' and upstream regions of genes.

25   Genomic libraries are preferred for obtaining introns and extending 5' sequences.

For hybridization techniques, a partial sequence may be labeled (*e.g.*, by nick-translation or end-labeling with ^{32}P) using well known techniques. A bacterial or bacteriophage library may then be screened by hybridizing filters containing denatured bacterial colonies (or lawns containing phage plaques) with the labeled probe (*see, e.g.,*

30   Sambrook et al., *Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989). Hybridizing colonies or plaques are

selected and expanded, and the DNA is isolated for further analysis. Clones may be analyzed to determine the amount of additional sequence by, for example, PCR using a primer from the partial sequence and a primer from the vector. Restriction maps and partial sequences may be generated to identify one or more overlapping clones. A full

5   length cDNA molecule can be generated by ligating suitable fragments, using well known techniques.

Alternatively, there are numerous amplification techniques for obtaining a full length coding sequence from a partial cDNA sequence. Within such techniques, amplification is generally performed via PCR. One such technique is known as "rapid

10   amplification of cDNA ends" or RACE. This technique involves the use of an internal primer and an external primer, which hybridizes to a polyA region or vector sequence, to identify sequences that are 5' and 3' of a known sequence. Any of a variety of commercially available kits may be used to perform the amplification step. Primers may be designed using, for example, software well known in the art. Primers are

15   preferably 17-32 nucleotides in length, have a GC content of at least 40% and anneal to the target sequence at temperatures of about 54°C to 72°C. The amplified region may be sequenced as described above, and overlapping sequences assembled into a contiguous sequence.

A cDNA sequence encoding DSP-16 is provided in Figure 1 (SEQ ID

20   NO:1), and the predicted amino acid sequence is provided in Figure 2 (SEQ ID NO:2). A cDNA sequence encoding a DSP-16 alternate form is provided in Figure 3 (SEQ ID NO:20), and the predicted amino acid sequence, which includes the DSP-16 active site VHCLAGISRS (SEQ ID NO:16), is provided in Figure 4 (SEQ ID NO:21). Sequence information immediately adjacent to this site was used to design 5' and 3' RACE

25   reactions with human thymus or human brain, skeletal muscle and testis cDNA to identify a protein of 665 amino acids (Fig. 2) encoded by 1995 base pairs. This protein is referred to as dual specificity phosphatase-3, or DSP-16. This approach also permitted identification of a DSP-16 alternate form protein of 517 amino acids (Fig. 4) encoded by 1551 base pairs which, according to non-limiting theory, may be the product

30   of alternate splicing at the polynucleotide level. DSP-16 shows significant homology to

other MAP-kinase phosphatases, as shown by the sequence comparison presented in
Figure 5.

DSP-16 (or DSP-16 alternate form) polynucleotide variants may
generally be prepared by any method known in the art, including, for example, solid

5   phase chemical synthesis. Modifications in a polynucleotide sequence may also be
introduced using standard mutagenesis techniques, such as oligonucleotide-directed
site-specific mutagenesis. Alternatively, RNA molecules may be generated by *in vitro*
or *in vivo* transcription of DNA sequences encoding DSP-16, or a portion thereof,
provided that the DNA is incorporated into a vector with a suitable RNA polymerase

10   promoter (such as T7 or SP6). Certain polynucleotides may be used to prepare an
encoded polypeptide, as described herein. In addition, or alternatively, a polynucleotide
may be administered to a patient such that the encoded polypeptide is generated *in vivo*.

A polynucleotide that is complementary to at least a portion of a coding
sequence (*e.g.*, an antisense polynucleotide or a ribozyme) may also be used as a probe

15   or primer, or to modulate gene expression. Identification of oligonucleotides and
ribozymes for use as antisense agents, and DNA encoding genes for their targeted
delivery, involve methods well known in the art. For example, the desirable properties,
lengths and other characteristics of such oligonucleotides are well known. Antisense
oligonucleotides are typically designed to resist degradation by endogenous nucleolytic

20   enzymes by using such linkages as: phosphorothioate, methylphosphonate, sulfone,
sulfate, ketyl, phosphorodithioate, phosphoramidate, phosphate esters, and other such
linkages (*see, e.g.*, Agrwal et al., *Tetrahedron Lett. 28*:3539-3542 (1987); Miller et al.,
·*J. Am. Chem. Soc. 93*:6657-6665 (1971); Stec et al., *Tetrahedron Lett. 26*:2191-2194
(1985); Moody et al., *Nucl. Acids Res. 12*:4769-4782 (1989); Uznanski et al., *Nucl.*

25   *Acids Res.* (1989); Letsinger et al., *Tetrahedron 40*:137-143 (1984); Eckstein, *Annu.*
Rev. Biochem. 54:367-402 (1985); Eckstein, *Trends Biol. Sci. 14*:97-100 (1989); Stein
In: *Oligodeoxynucleotides. Antisense Inhibitors of Gene Expression*, Cohen, Ed,
Macmillan Press, London, pp. 97-117 (1989); Jager et al., *Biochemistry 27*:7237-7246
(1988)).

30   Antisense polynucleotides are oligonucleotides that bind in a sequence-
specific manner to nucleic acids, such as mRNA or DNA. When bound to mRNA that

has complementary sequences, antisense prevents translation of the mRNA (*see, e.g.,*
U.S. Patent No. 5,168,053 to Altman et al.; U.S. Patent No. 5,190,931 to Inouye, U.S.
Patent No. 5,135,917 to Burch; U.S. Patent No. 5,087,617 to Smith and Clusel et al.
(1993) *Nucl. Acids Res. 21*:3405-3411, which describes dumbbell antisense
5   oligonucleotides). Triplex molecules refer to single DNA strands that bind duplex
DNA forming a colinear triplex molecule, thereby preventing transcription (*see, e.g.,*
U.S. Patent No. 5,176,996 to Hogan et al., which describes methods for making
synthetic oligonucleotides that bind to target sites on duplex DNA).

        Particularly useful antisense nucleotides and triplex molecules are
10   molecules that are complementary to or bind the sense strand of DNA or mRNA that
encodes a DSP-16 or a DSP-16 alternate form polypeptide or a protein mediating any
other process related to expression of endogenous DSP-16 (or DSP-16 alternate form),
such that inhibition of translation of mRNA encoding the DSP-16 (or DSP-16 alternate
form) polypeptide is effected. cDNA constructs that can be transcribed into antisense
15   RNA may also be introduced into cells or tissues to facilitate the production of antisense
RNA. Antisense technology can be used to control gene expression through
interference with binding of polymerases, transcription factors or other regulatory
molecules (*see* Gee et al., *In* Huber and Carr, *Molecular and Immunologic Approaches*,
Futura Publishing Co. (Mt. Kisco, NY; 1994)). Alternatively, an antisense molecule
20   may be designed to hybridize with a control region of a DSP-16 gene (*e.g.,* promoter,
enhancer or transcription initiation site), and block transcription of the gene; or to block
translation by inhibiting binding of a transcript to ribosomes.

        The present invention also contemplates DSP-16- (or DSP-16 alternate
form) specific ribozymes. A ribozyme is an RNA molecule that specifically cleaves
25   RNA substrates, such as mRNA, resulting in specific inhibition or interference with
cellular gene expression. There are at least five known classes of ribozymes involved in
the cleavage and/or ligation of RNA chains. Ribozymes can be targeted to any RNA
transcript and can catalytically cleave such transcripts (*see, e.g.,* U.S. Patent No.
5,272,262; U.S. Patent No. 5,144,019; and U.S. Patent Nos. 5,168,053, 5,180,818,
30   5,116,742 and 5,093,246 to Cech et al.). Any DSP-16 (or DSP-16 alternate form)
mRNA-specific ribozyme, or a nucleic acid encoding such a ribozyme, may be

delivered to a host cell to effect inhibition of DSP-16 gene expression. Ribozymes may therefore be delivered to the host cells by DNA encoding the ribozyme linked to a eukaryotic promoter, such as a eukaryotic viral promoter, such that upon introduction into the nucleus, the ribozyme will be directly transcribed.

5   Any polynucleotide may be further modified to increase stability *in vivo*. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends; the use of phosphorothioate or 2' O-methyl rather than phosphodiester linkages in the backbone; and/or the inclusion of nontraditional bases such as inosine, queosine and wybutosine, as well as acetyl- methyl-, thio- and

10   other modified forms of adenine, cytidine, guanine, thymine and uridine.

Nucleotide sequences as described herein may be joined to a variety of other nucleotide sequences using established recombinant DNA techniques. For example, a polynucleotide may be cloned into any of a variety of cloning vectors, including plasmids, phagemids, lambda phage derivatives and cosmids. Vectors of

15   particular interest include expression vectors, replication vectors, probe generation vectors and sequencing vectors. In general, a suitable vector contains an origin of replication functional in at least one organism, convenient restriction endonuclease sites and one or more selectable markers. Other elements will depend upon the desired use, and will be apparent to those having ordinary skill in the art.

20   Within certain embodiments, polynucleotides may be formulated so as to permit entry into a cell of a mammal, and expression therein. Such formulations are particularly useful for therapeutic purposes, as described below. Those having ordinary skill in the art will appreciate that there are many ways to achieve expression of a polynucleotide in a target cell, and any suitable method may be employed. For

25   example, a polynucleotide may be incorporated into a viral vector using well known techniques. A viral vector may additionally transfer or incorporate a gene for a selectable marker (to aid in the identification or selection of transduced cells) and/or a targeting moiety, such as a gene that encodes a ligand for a receptor on a specific target cell, to render the vector target specific. Targeting may also be accomplished using an

30   antibody, by methods known to those having ordinary skill in the art.

Other formulations for therapeutic purposes include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. A preferred colloidal system for use as a delivery vehicle *in vitro* and *in*
5 *vivo* is a liposome (*i.e.,* an artificial membrane vesicle). The preparation and use of such systems is well known in the art.

Within other aspects, a DSP-16 promoter may be isolated using standard techniques. The present invention provides nucleic acid molecules comprising such a promoter sequence or one or more cis- or trans-acting regulatory elements thereof.
10 Such regulatory elements may enhance or suppress expression of DSP-16 (or DSP-16 alternate form). A 5' flanking region may be generated using standard techniques, based on the genomic sequence provided herein. If necessary, additional 5' sequences may be generated using PCR-based or other standard methods. The 5' region may be subcloned and sequenced using standard methods. Primer extension and/or RNase protection
15 analyses may be used to verify the transcriptional start site deduced from the cDNA.

To define the boundary of the promoter region, putative promoter inserts of varying sizes may be subcloned into a heterologous expression system containing a suitable reporter gene without a promoter or enhancer. Suitable reporter genes may include genes encoding luciferase, beta-galactosidase, chloramphenicol acetyl
20 transferase, secreted alkaline phosphatase or the Green Fluorescent Protein gene. Suitable expression systems are well known and may be prepared using well known techniques or obtained commercially. Internal deletion constructs may be generated using unique internal restriction sites or by partial digestion of non-unique restriction sites. Constructs may then be transfected into cells that display high levels of DSP-16
25 expression. In general, the construct with the minimal 5' flanking region showing the highest level of expression of reporter gene is identified as the promoter. Such promoter regions may be linked to a reporter gene and used to evaluate agents for the ability to modulate DSP-16 transcription.

Once a functional promoter is identified, cis- and trans-acting elements
30 may be located. Cis-acting sequences may generally be identified based on homology to previously characterized transcriptional motifs. Point mutations may then be

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generated within the identified sequences to evaluate the regulatory role of such sequences. Such mutations may be generated using site-specific mutagenesis techniques or a PCR-based strategy. The altered promoter is then cloned into a reporter gene expression vector, as described above, and the effect of the mutation on reporter
5   gene expression is evaluated.

The present invention also contemplates the use of allelic variants of DSP-16 (or DSP-16 alternate form), as well as DSP-16 sequences from other organisms. Such sequences may generally be identified based upon similarity to the sequences provided herein (e.g., using hybridization techniques) and based upon the
10   presence of DSP-16 activity, using an assay provided herein.

In general, polypeptides and polynucleotides as described herein are isolated. An "isolated" polypeptide or polynucleotide is one that is removed from its original environment. For example, a naturally-occurring protein is isolated if it is separated from some or all of the coexisting materials in the natural system. Preferably,
15   such polypeptides are at least about 90% pure, more preferably at least about 95% pure and most preferably at least about 99% pure. A polynucleotide is considered to be isolated if, for example, it is cloned into a vector that is not a part of the natural environment.

ASSAYS FOR DETECTING DSP-16 ACTIVITY

20   According to the present invention, substrates of DSP-16 (or DSP-16 alternate form) may include full length tyrosine phosphorylated proteins and polypeptides as well as fragments (e.g., portions), derivatives or analogs thereof that can be phosphorylated at a tyrosine residue and that may, in certain preferred embodiments, also be able to undergo phosphorylation at a serine or a threonine residue. Such
25   fragments, derivatives and analogs include any naturally occurring or artificially engineered DSP-16 substrate polypeptide that retains at least the biological function of interacting with a DSP-16 (or DSP-16 alternate form) as provided herein, for example by forming a complex with a DSP-16 (or DSP-16 alternate form). A fragment, derivative or analog of a DSP-16 substrate polypeptide, including substrates that are
30   fusion proteins, may be (i) one in which one or more of the amino acid residues are

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substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue), and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the substrate polypeptide is

5 fused with another compound, such as a compound to increase the half-life of the polypeptide (e.g., polyethylene glycol) or a detectable moiety such as a reporter molecule, or (iv) one in which additional amino acids are fused to the substrate polypeptide, including amino acids that are employed for purification of the substrate polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are

10 deemed to be within the scope of those skilled in the art. In preferred embodiment, a MAP-kinase polypeptide is a substrate for use as provided herein.

DSP-16 (or DSP-16 alternate form) polypeptide variants may be tested for DSP-16 activity using any suitable assay for MAP-kinase phosphatase activity. Such assays may be performed in vitro or within a cell-based assay. For example, a

15 MAP-kinase may be obtained in inactive form from Upstate Biotechnology (Lake Placid, NY; catalog number 14-198), for use as a DSP-16 substrate as provided herein. Phosphorylation of the MAP-kinase can be performed using well known techniques (such as those described by Zheng and Guan, J. Biol. Chem. 268:16116-16119, 1993) using the MAP-kinase kinase MEK-1 (available from Upstate Biotechnology; cat. no.

20 14-206).

For example, $[^{32}P]$-radiolabeled substrate (e.g., MAP-kinase) may be used for the kinase reaction, resulting in radiolabeled, activated MAP-kinase. A DSP-16 (or DSP-16 alternate form) polypeptide may then be tested for the ability to dephosphorylate an activated MAP-kinase by contacting the DSP-16 (or DSP-16

25 alternate form) polypeptide with the MAP-kinase under suitable conditions (e.g., Tris, pH 7.5, 1 mM EDTA, 1 mM dithiothreitol, 1 mg/mL bovine serum albumin for 10 minutes at 30°C; or as described by Zheng and Guan, J. Biol. Chem. 268:16116-16119, 1993). Dephosphorylation of the MAP-kinase may be detected using any of a variety of assays, such as a coupled kinase assay (evaluating phosphorylation of a MAP-kinase

30 substrate using any assay generally known in the art) or directly, based on (1) the loss of radioactive phosphate groups (e.g., by gel electrophoresis, followed by

autoradiography); (2) the shift in electrophoretic mobility following dephosphorylation; (3) the loss of reactivity with an antibody specific for phosphotyrosine or phosphothreonine; or (4) a phosphoamino acid analysis of the MAP-kinase. Certain assays may generally be performed as described by Ward et al., *Nature 367*:651-654, 1994 or Alessi et al., *Oncogene 8*:2015-2020, 1993. In general, contact of 500 pg - 50 ng of DSP-16 polypeptide with 100ng - 100 µg activated MAP-kinase should result in a detectable dephosphorylation of the MAP-kinase, typically within 20-30 minutes. Within certain embodiments, 0.01 - 10 units/mL (preferably about 0.1 units/mL, where a unit is an amount sufficient to dephosphorylate 1 nmol substrate per minute) DSP-16 polypeptide may be contacted with 0.1 - 10 µM (preferably about 1 µM) activated MAP-kinase to produce a detectable dephosphorylation of a MAP-kinase. Preferably, a DSP-16 polypeptide results in a dephosphorylation of a MAP-kinase or a phosphorylated substrate (such as a tyrosine- and/or serine-phosphorylated peptide) that is at least as great as the dephosphorylation observed in the presence of a comparable amount of native human DSP-16. It will be apparent that other substrates identified using a substrate trapping mutant as described herein may be substituted for the MAP-kinase within such assays.

ANTIBODIES AND ANTIGEN-BINDING FRAGMENTS

Also contemplated by the present invention are peptides, polypeptides, and other non-peptide molecules that specifically bind to a DSP-16 (or DSP-16 alternate form). As used herein, a molecule is said to "specifically bind" to a DSP-16 (or DSP-16 alternate form) if it reacts at a detectable level with DSP-16 (or DSP-16 alternate form), but does not react detectably with peptides containing an unrelated sequence, or a sequence of a different phosphatase. Preferred binding molecules include antibodies, which may be, for example, polyclonal, monoclonal, single chain, chimeric, anti-idiotypic, or CDR-grafted immunoglobulins, or fragments thereof, such as proteolytically generated or recombinantly produced immunoglobulin F(ab')$_2$, Fab, Fv, and Fd fragments. Certain preferred antibodies are those antibodies that inhibit or block DSP-16 activity within an *in vitro* assay, as described herein. Binding properties of an antibody to DSP-16 may generally be assessed using immunodetection methods

including, for example, an enzyme-linked immunosorbent assay (ELISA), immunoprecipitation, immunoblotting and the like, which may be readily performed by those having ordinary skill in the art.

5   Methods well known in the art may be used to generate antibodies, polyclonal antisera or monoclonal antibodies that are specific for a DSP-16 (or DSP-16 alternate form). Antibodies also may be produced as genetically engineered immunoglobulins (Ig) or Ig fragments designed to have desirable properties. For example, by way of illustration and not limitation, antibodies may include a recombinant IgG that is a chimeric fusion protein having at least one variable (V) region

10   domain from a first mammalian species and at least one constant region domain from a second, distinct mammalian species. Most commonly, a chimeric antibody has murine variable region sequences and human constant region sequences. Such a murine/human chimeric immunoglobulin may be "humanized" by grafting the complementarity determining regions (CDRs) derived from a murine antibody, which confer binding

15   specificity for an antigen, into human-derived V region framework regions and human-derived constant regions. Fragments of these molecules may be generated by proteolytic digestion, or optionally, by proteolytic digestion followed by mild reduction of disulfide bonds and alkylation. Alternatively, such fragments may also be generated by recombinant genetic engineering techniques.

20   As used herein, an antibody is said to be "immunospecific" or to "specifically bind" a DSP-16 (or DSP-16 alternate form) polypeptide if it reacts at a detectable level with DSP-16 (or DSP-16 alternate form), preferably with an affinity constant, K_a, of greater than or equal to about 10^4 M^{-1}, more preferably of greater than or equal to about 10^5 M^{-1}, more preferably of greater than or equal to about 10^6 M^{-1},

25   and still more preferably of greater than or equal to about 10^7 M^{-1}. Affinities of binding partners or antibodies can be readily determined using conventional techniques, for example, those described by Scatchard et al. (*Ann. N.Y. Acad. Sci. USA 51*:660 (1949)) or by surface plasmon resonance (BIAcore, Biosensor, Piscataway, NJ). *See, e.g.,* Wolff et al., *Cancer Res. 53*:2560-2565 (1993).

30   Antibodies may generally be prepared by any of a variety of techniques known to those having ordinary skill in the art. *See, e.g.,* Harlow et al., *Antibodies: A*

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Laboratory Manual, Cold Spring Harbor Laboratory (1988). In one such technique, an animal is immunized with DSP-16 as an antigen to generate polyclonal antisera. Suitable animals include, for example, rabbits, sheep, goats, pigs, cattle, and may also include smaller mammalian species, such as mice, rats, and hamsters, or other species.

5   An immunogen may be comprised of cells expressing DSP-16 (or DSP-16 alternate form), purified or partially purified DSP-16 (or DSP-16 alternate form) polypeptides or variants or fragments (*e.g.*, peptides) thereof, or DSP-16 peptides. DSP-16 peptides may be generated by proteolytic cleavage or may be chemically synthesized. For instance, nucleic acid sequences encoding DSP-16 (or DSP-16 alternate form) polypeptides are provided herein, such that those skilled in the art may routinely prepare these polypeptides for use as immunogens. Polypeptides or peptides useful for immunization may also be selected by analyzing the primary, secondary, and tertiary structure of DSP-16 according to methods known to those skilled in the art, in order to determine amino acid sequences more likely to generate an antigenic response in a host animal. *See, e.g.*, Novotny, 1991 *Mol. Immunol.* 28:201-207; Berzofsky, 1985 *Science* 229:932-40.

          Preparation of the immunogen for injection into animals may include covalent coupling of the DSP-16 (or DSP-16 alternate form) polypeptide (or variant or fragment thereof), to another immunogenic protein, for example, a carrier protein such as keyhole limpet hemocyanin (KLH) or bovine serum albumin (BSA). In addition, the DSP-16 peptide, polypeptide, or DSP-16-expressing cells to be used as immunogen may be emulsified in an adjuvant. *See, e.g.*, Harlow et al., *Antibodies: A Laboratory Manual,* Cold Spring Harbor Laboratory (1988). In general, after the first injection, animals receive one or more booster immunizations according to a preferred schedule that may vary according to, *inter alia*, the antigen, the adjuvant (if any) and/or the particular animal species. The immune response may be monitored by periodically bleeding the animal, separating the sera out of the collected blood, and analyzing the sera in an immunoassay, such as an ELISA or Ouchterlony diffusion assay, or the like, to determine the specific antibody titer. Once an antibody titer is established, the animals may be bled periodically to accumulate the polyclonal antisera. Polyclonal antibodies that bind specifically to the DSP-16 polypeptide or peptide may then be

purified from such antisera, for example, by affinity chromatography using protein A, or the DSP-16 polypeptide, immobilized on a suitable solid support.

Monoclonal antibodies that specifically bind to DSP-16 (or DSP-16 alternate form) polypeptides or fragments or variants thereof, and hybridomas, which

5   are immortal eukaryotic cell lines, that produce monoclonal antibodies having the desired binding specificity, may also be prepared, for example, using the technique of Kohler and Milstein (*Nature,* 256:495-497; 1976, *Eur. J. Immunol.* 6:511-519 (1975)) and improvements thereto. An animal—for example, a rat, hamster, or preferably mouse—is immunized with a DSP-16 immunogen prepared as described above.

10   Lymphoid cells that include antibody-forming cells, typically spleen cells, are obtained from an immunized animal and may be immortalized by fusion with a drug-sensitized myeloma (*e.g.,* plasmacytoma) cell fusion partner, preferably one that is syngeneic with the immunized animal and that optionally has other desirable properties (*e.g.,* inability to express endogenous Ig gene products). The lymphoid (*e.g.,* spleen) cells and the

15   myeloma cells may be combined for a few minutes with a membrane fusion-promoting agent, such as polyethylene glycol or a nonionic detergent, and then plated at low density on a selective medium that supports the growth of hybridoma cells, but not unfused myeloma cells. A preferred selection media is HAT (hypoxanthine, aminopterin, thymidine). After a sufficient time, usually about one to two weeks,

20   colonies of cells are observed. Single colonies are isolated, and antibodies produced by the cells may be tested for binding activity to the DSP-16 polypeptide, or variant or fragment thereof. Hybridomas producing monoclonal antibodies with high affinity and specificity for a DSP-16 antigen are preferred. Hybridomas that produce monoclonal antibodies that specifically bind to a DSP-16 polypeptide or variant or fragment thereof

25   are therefore contemplated by the present invention.

Monoclonal antibodies may be isolated from the supernatants of hybridoma cultures. An alternative method for production of a murine monoclonal antibody is to inject the hybridoma cells into the peritoneal cavity of a syngeneic mouse, for example, a mouse that has been treated (*e.g.,* pristane-primed) to promote formation

30   of ascites fluid containing the monoclonal antibody. Contaminants may be removed from the subsequently (usually within 1-3 weeks) harvested ascites fluid by

conventional techniques, such as chromatography, gel filtration, precipitation, extraction, or the like. For example, antibodies may be purified by affinity chromatography using an appropriate ligand selected based on particular properties of the monoclonal antibody (e.g., heavy or light chain isotype, binding specificity, etc.).

5   Examples of a suitable ligand, immobilized on a solid support, include Protein A, Protein G, an anti-constant region (light chain or heavy chain) antibody, an anti-idiotype antibody and a DSP-16 polypeptide or fragment or variant thereof.

Human monoclonal antibodies may be generated by any number of techniques with which those having ordinary skill in the art will be familiar. Such

10   methods include but are not limited to, Epstein Barr Virus (EBV) transformation of human peripheral blood cells (e.g., containing B lymphocytes), in vitro immunization of human B cells, fusion of spleen cells from immunized transgenic mice carrying human immunoglobulin genes inserted by yeast artificial chromosomes (YAC), isolation from human immunoglobulin V region phage libraries, or other procedures as known in the

15   art and based on the disclosure herein.

For example, one method for generating human monoclonal antibodies includes immortalizing human peripheral blood cells by EBV transformation. See, e.g., U.S. Patent No. 4,464,456. An immortalized cell line producing a monoclonal antibody that specifically binds to a DSP-16 polypeptide (or a variant or fragment thereof) can be

20   identified by immunodetection methods as provided herein, for example, an ELISA, and then isolated by standard cloning techniques. Another method to generate human monoclonal antibodies, in vitro immunization, includes priming human splenic B cells with antigen, followed by fusion of primed B cells with a heterohybrid fusion partner. See, e.g., Boerner et al., 1991 J. Immunol. 147:86-95.

25   Still another method for the generation of human DSP-16-specific monoclonal antibodies and polyclonal antisera for use in the present invention relates to transgenic mice. See, e.g., U.S. Patent No. 5,877,397; Bruggemann et al., 1997 Curr. Opin. Biotechnol. 8:455-58; Jakobovits et al., 1995 Ann. N. Y. Acad. Sci. 764:525-35. In these mice, human immunoglobulin heavy and light chain genes have been

30   artificially introduced by genetic engineering in germline configuration, and the endogenous murine immunoglobulin genes have been inactivated. See, e.g.,

Bruggemann et al., 1997 *Curr. Opin. Biotechnol.* 8:455-58. For example, human immunoglobulin transgenes may be mini-gene constructs, or transloci on yeast artificial chromosomes, which undergo B cell-specific DNA rearrangement and hypermutation in the mouse lymphoid tissue. *See*, Bruggemann et al., 1997 *Curr. Opin. Biotechnol.*
5 8:455-58. Human monoclonal antibodies specifically binding to DSP-16 may be obtained by immunizing the transgenic animals, fusing spleen cells with myeloma cells, selecting and then cloning cells producing antibody, as described above. Polyclonal sera containing human antibodies may also be obtained from the blood of the immunized animals.

10   Chimeric antibodies, specific for a DSP-16, including humanized antibodies, may also be generated according to the present invention. A chimeric antibody has at least one constant region domain derived from a first mammalian species and at least one variable region domain derived from a second, distinct mammalian species. *See, e.g.,* Morrison et al., 1984, *Proc. Natl. Acad. Sci. USA,*
15 81:6851-55. In preferred embodiments, a chimeric antibody may be constructed by cloning the polynucleotide sequence that encodes at least one variable region domain derived from a non-human monoclonal antibody, such as the variable region derived from a murine, rat, or hamster monoclonal antibody, into a vector containing a nucleic acid sequence that encodes at least one human constant region. *See, e.g.,* Shin et al.,
20 1989 *Methods Enzymol.* 178:459-76; Walls et al., 1993 *Nucleic Acids Res.* 21:2921-29. By way of example, the polynucleotide sequence encoding the light chain variable region of a murine monoclonal antibody may be inserted into a vector containing a nucleic acid sequence encoding the human kappa light chain constant region sequence. In a separate vector, the polynucleotide sequence encoding the heavy chain variable
25 region of the monoclonal antibody may be cloned in frame with sequences encoding the human IgG1 constant region. The particular human constant region selected may depend upon the effector functions desired for the particular antibody (*e.g.*, complement fixing, binding to a particular Fc receptor, etc.). Another method known in the art for generating chimeric antibodies is homologous recombination (*e.g.*, U.S. Patent
30 No. 5,482,856). Preferably, the vectors will be transfected into eukaryotic cells for stable expression of the chimeric antibody.

A non-human/human chimeric antibody may be further genetically engineered to create a "humanized" antibody. Such a humanized antibody may comprise a plurality of CDRs derived from an immunoglobulin of a non-human mammalian species, at least one human variable framework region, and at least one

5   human immunoglobulin constant region. Humanization may in certain embodiments provide an antibody that has decreased binding affinity for a DSP-16 when compared, for example, with either a non-human monoclonal antibody from which a DSP-16 binding variable region is obtained, or a chimeric antibody having such a V region and at least one human C region, as described above. Useful strategies for designing

10   humanized antibodies may therefore include, for example by way of illustration and not limitation, identification of human variable framework regions that are most homologous to the non-human framework regions of the chimeric antibody. Without wishing to be bound by theory, such a strategy may increase the likelihood that the humanized antibody will retain specific binding affinity for a DSP-16, which in some

15   preferred embodiments may be substantially the same affinity for a DSP-16 polypeptide or variant or fragment thereof, and in certain other preferred embodiments may be a greater affinity for DSP-16. See, e.g., Jones et al., 1986 Nature 321:522-25; Riechmann et al., 1988 Nature 332:323-27. Designing such a humanized antibody may therefore include determining CDR loop conformations and structural determinants of the non-

20   human variable regions, for example, by computer modeling, and then comparing the CDR loops and determinants to known human CDR loop structures and determinants. See, e.g., Padlan et al., 1995 FASEB 9:133-39; Chothia et al., 1989 Nature, 342:377-383. Computer modeling may also be used to compare human structural templates selected by sequence homology with the non-human variable regions. See, e.g.,

25   Bajorath et al., 1995 Ther. Immunol. 2:95-103; EP-0578515-A3. If humanization of the non-human CDRs results in a decrease in binding affinity, computer modeling may aid in identifying specific amino acid residues that could be changed by site-directed or other mutagenesis techniques to partially, completely or supra-optimally (i.e., increase to a level greater than that of the non-humanized antibody) restore affinity. Those

30   having ordinary skill in the art are familiar with these techniques, and will readily appreciate numerous variations and modifications to such design strategies.

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conditions, are toxic to cells. As used herein, a toxin polypeptide for inclusion in an immunoglobulin-toxin fusion protein may be any polypeptide capable of being introduced to a cell in a manner that compromises cell survival, for example, by directly interfering with a vital function or by inducing apoptosis. Toxins thus may include, for

5   example, ribosome-inactivating proteins, such as *Pseudomonas aeruginosa* exotoxin A, plant gelonin, bryodin from *Bryonia dioica*, or the like. *See, e.g.*, Thrush et al., 1996 *Annu. Rev. Immunol. 14*:49-71; Frankel et al., 1996 *Cancer Res. 56*:926-32. Numerous other toxins, including chemotherapeutic agents, anti-mitotic agents, antibiotics, inducers of apoptosis (or "apoptogens", see, *e.g.*, Green and Reed, 1998, *Science*

10   *281*:1309-1312), or the like, are known to those familiar with the art, and the examples provided herein are intended to be illustrative without limiting the scope and spirit of the invention.

             The sFv may, in certain embodiments, be fused to peptide or polypeptide domains that permit detection of specific binding between the fusion protein and

15   antigen (*e.g.*, a DSP-16). For example, the fusion polypeptide domain may be an affinity tag polypeptide. Binding of the sFv fusion protein to a binding partner (*e.g.*, a DSP-16) may therefore be detected using an affinity polypeptide or peptide tag, such as an avidin, streptavidin or a His (*e.g.*, polyhistidine) tag, by any of a variety of techniques with which those skilled in the art will be familiar. Detection techniques may also

20   include, for example, binding of an avidin or streptavidin fusion protein to biotin or to a biotin mimetic sequence (*see, e.g.*, Luo et al., 1998 *J. Biotechnol. 65*:225 and references cited therein), direct covalent modification of a fusion protein with a detectable moiety (*e.g.*, a labeling moiety), non-covalent binding of the fusion protein to a specific labeled reporter molecule, enzymatic modification of a detectable substrate by a fusion protein

25   that includes a portion having enzyme activity, or immobilization (covalent or non-covalent) of the fusion protein on a solid-phase support.

             The sFv fusion protein of the present invention, comprising a DSP-16-specific immunoglobulin-derived polypeptide fused to another polypeptide such as an effector peptide having desirable affinity properties, may therefore include, for example,

30   a fusion protein wherein the effector peptide is an enzyme such as glutathione-S-transferase. As another example, sFv fusion proteins may also comprise a DSP-16-

specific Ig polypeptide fused to a *Staphylococcus aureus* protein A polypeptide; protein A encoding nucleic acids and their use in constructing fusion proteins having affinity for immunoglobulin constant regions are disclosed generally, for example, in U.S. Patent 5,100,788. Other useful affinity polypeptides for construction of sFv fusion

5   proteins may include streptavidin fusion proteins, as disclosed, for example, in WO 89/03422; U.S. 5,489,528; U.S. 5,672,691; WO 93/24631; U.S. 5,168,049; U.S. 5,272,254 and elsewhere, and avidin fusion proteins (see, *e.g.,* EP 511,747). As provided herein, sFv polypeptide sequences may be fused to fusion polypeptide sequences, including effector protein sequences, that may include full length fusion

10   polypeptides and that may alternatively contain variants or fragments thereof.

An additional method for selecting antibodies that specifically bind to a DSP-16 polypeptide or variant or fragment thereof is by phage display. *See, e.g.,* Winter et al., 1994 *Annul. Rev. Immunol.* 12:433-55; Burton et al., 1994 *Adv. Immunol.* 57:191-280. Human or murine immunoglobulin variable region gene

15   combinatorial libraries may be created in phage vectors that can be screened to select Ig fragments (Fab, Fv, sFv, or multimers thereof) that bind specifically to a DSP-16 polypeptide or variant or fragment thereof. *See, e.g.,* U.S. Patent No. 5,223,409; Huse et al., 1989 *Science* 246:1275-81; Kang et al., 1991 *Proc. Natl. Acad. Sci. USA 88:*4363-66; Hoogenboom et al., 1992 *J. Molec. Biol. 227:*381-388; Schlebusch et al., 1997

20   *Hybridoma 16:*47-52 and references cited therein. For example, a library containing a plurality of polynucleotide sequences encoding Ig variable region fragments may be inserted into the genome of a filamentous bacteriophage, such as M13 or a variant thereof, in frame with the sequence encoding a phage coat protein, for instance, gene III or gene VIII of M13, to create an M13 fusion protein. A fusion protein may be a fusion

25   of the coat protein with the light chain variable region domain and/or with the heavy chain variable region domain.

According to certain embodiments, immunoglobulin Fab fragments may also be displayed on the phage particle, as follows. Polynucleotide sequences encoding Ig constant region domains may be inserted into the phage genome in frame with a coat

30   protein. The phage coat fusion protein may thus be fused to an Ig light chain or heavy chain fragment (Fd). For example, from a human Ig library, the polynucleotide

sequence encoding the human kappa constant region may be inserted into a vector in frame with the sequence encoding at least one of the phage coat proteins. Additionally or alternatively, the polynucleotide sequence encoding the human IgG1 CH1 domain may be inserted in frame with the sequence encoding at least one other of the phage coat

5   proteins. A plurality of polynucleotide sequences encoding variable region domains (e.g., derived from a DNA library) may then be inserted into the vector in frame with the constant region-coat protein fusions, for expression of Fab fragments fused to a bacteriophage coat protein.

Phage that display an Ig fragment (e.g., an Ig V-region or Fab) that binds

10   to a DSP-16 polypeptide may be selected by mixing the phage library with DSP-16 or a variant or a fragment thereof, or by contacting the phage library with a DSP-16 polypeptide immobilized on a solid matrix under conditions and for a time sufficient to allow binding. Unbound phage are removed by a wash, which typically may be a buffer containing salt (e.g., NaCl) at a low concentration, preferably with less than 100 mM

15   NaCl, more preferably with less than 50 mM NaCl, most preferably with less than 10 mM NaCl, or, alternatively, a buffer containing no salt. Specifically bound phage are then eluted with an NaCl-containing buffer, for example, by increasing the salt concentration in a step-wise manner. Typically, phage that bind the DSP-16 with higher affinity will require higher salt concentrations to be released. Eluted phage may be

20   propagated in an appropriate bacterial host, and generally, successive rounds of DSP-16 binding and elution can be repeated to increase the yield of phage expressing DSP-16 specific immunoglobulin. Combinatorial phage libraries may also be used for humanization of non-human variable regions. See, e.g., Rosok et al., 1996 J. Biol. Chem. 271:22611-18; Rader et al., 1998 Proc. Natl. Acad. Sci. USA 95:8910-15. The

25   DNA sequence of the inserted immunoglobulin gene in the phage so selected may be determined by standard techniques. See, Sambrook et al., 1989 Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press. The affinity selected Ig-encoding sequence may then be cloned into another suitable vector for expression of the Ig fragment or, optionally, may be cloned into a vector containing Ig constant regions, for

30   expression of whole immunoglobulin chains.

Phage display techniques may also be used to select polypeptides, peptides or single chain antibodies that bind to DSP-16. For examples of suitable vectors having multicloning sites into which candidate nucleic acid molecules (*e.g.*, DNA) encoding such peptides or antibodies may be inserted, *see, e.g.*, McLafferty et al.,

5   *Gene 128*:29-36, 1993; Scott et al., 1990 *Science 249*:386-390; Smith et al., 1993 *Methods Enzymol. 217*:228-257; Fisch et al., 1996, *Proc. Natl. Acad. Sci. USA* 93:7761-66. The inserted DNA molecules may comprise randomly generated sequences, or may encode variants of a known peptide or polypeptide domain that specifically binds to a DSP-16 polypeptide, or variant or fragment thereof, as provided herein. Generally, the

10   nucleic acid insert encodes a peptide of up to 60 amino acids, more preferably a peptide of 3 to 35 amino acids, and still more preferably a peptide of 6 to 20 amino acids. The peptide encoded by the inserted sequence is displayed on the surface of the bacteriophage. Phage expressing a binding domain for a DSP-16 polypeptide may be selected on the basis of specific binding to an immobilized DSP-16 polypeptide as

15   described above. As provided herein, well-known recombinant genetic techniques may be used to construct fusion proteins containing the fragment thereof. For example, a polypeptide may be generated that comprises a tandem array of two or more similar or dissimilar affinity selected DSP-16 binding peptide domains, in order to maximize binding affinity for DSP-16 of the resulting product.

20   In certain other embodiments, the invention contemplates DSP-16 specific antibodies that are multimeric antibody fragments. Useful methodologies are described generally, for example in Hayden et al. 1997, *Curr Opin. Immunol. 9*:201-12; Coloma et al., 1997 *Nat. Biotechnol. 15*:159-63). For example, multimeric antibody fragments may be created by phage techniques to form miniantibodies (U.S. Patent No.

25   5,910 573) or diabodies (Holliger et al., 1997, *Cancer Immunol. Immunother. 45*:128-130). Multimeric fragments may be generated that are multimers of a DSP-16-specific Fv, or that are bispecific antibodies comprising a DSP-16-specific Fv noncovalently associated with a second Fv having a different antigen specificity. *See, e.g.*, Koelemij et al., 1999 *J. Immunother. 22*:514-24. As another example, a multimeric antibody may

30   comprise a bispecific antibody having two single chain antibodies or Fab fragments. According to certain related embodiments, a first Ig fragment may be specific for a first

antigenic determinant on a DSP-16 polypeptide (or variant or fragment thereof), while a second Ig fragment may be specific for a second antigenic determinant of the DSP-16 polypeptide. Alternatively, in certain other related embodiments, a first immunoglobulin fragment may be specific for an antigenic determinant on a DSP-16

5 polypeptide or variant or fragment thereof, and a second immunoglobulin fragment may be specific for an antigenic determinant on a second, distinct (i.e., non-DSP-16) molecule. Also contemplated are bispecific antibodies that specifically bind DSP-16, wherein at least one antigen-binding domain is present as a fusion protein.

Introducing amino acid mutations into DSP-16-binding immunoglobulin

10 molecules may be useful to increase the specificity or affinity for DSP-16, or to alter an effector function. Immunoglobulins with higher affinity for DSP-16 may be generated by site-directed mutagenesis of particular residues. Computer assisted three-dimensional molecular modeling may be employed to identify the amino acid residues to be changed, in order to improve affinity for the DSP-16 polypeptide . See, e.g.,

15 Mountain et al., 1992, Biotechnol. Genet. Eng. Rev. 10: 1-142. Alternatively, combinatorial libraries of CDRs may be generated in M13 phage and screened for immunoglobulin fragments with improved affinity. See, e.g., Glaser et al., 1992, J. Immunol. 149:3903-3913; Barbas et al., 1994 Proc. Natl. Acad. Sci. USA 91:3809-13; U.S. Patent No. 5,792,456).

20 Effector functions may also be altered by site-directed mutagenesis. See, e.g., Duncan et al., 1988 Nature 332:563-64; Morgan et al., 1995 Immunology 86:319-24; Eghtedarzedeh-Kondri et al., 1997 Biotechniques 23:830-34. For example, mutation of the glycosylation site on the Fc portion of the immunoglobulin may alter the ability of the immunoglobulin to fix complement. See, e.g., Wright et al., 1997 Trends

25 Biotechnol. 15:26-32. Other mutations in the constant region domains may alter the ability of the immunoglobulin to fix complement, or to effect antibody-dependent cellular cytotoxicity. See, e.g., Duncan et al., 1988 Nature 332:563-64; Morgan et al., 1995 Immunology 86:319-24; Sensel et al., 1997 Mol. Immunol. 34:1019-29.

The nucleic acid molecules encoding an antibody or fragment thereof

30 that specifically binds DSP-16, as described herein, may be propagated and expressed according to any of a variety of well-known procedures for nucleic acid excision,

ligation, transformation and transfection. Thus, in certain embodiments expression of an antibody fragment may be preferred in a prokaryotic host, such as *Escherichia coli* (*see, e.g.*, Pluckthun et al., 1989 *Methods Enzymol. 178*:497-515). In certain other embodiments, expression of the antibody or a fragment thereof may be preferred in a

5   eukaryotic host cell, including yeast (*e.g.*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and *Pichia pastoris*), animal cells (including mammalian cells) or plant cells. Examples of suitable animal cells include, but are not limited to, myeloma, COS, CHO, or hybridoma cells. Examples of plant cells include tobacco, corn, soybean, and rice cells. By methods known to those having ordinary skill in the

10   art and based on the present disclosure, a nucleic acid vector may be designed for expressing foreign sequences in a particular host system, and then polynucleotide sequences encoding the DSP-16 binding antibody (or fragment thereof) may be inserted. The regulatory elements will vary according to the particular host.

A DSP-16-binding immunoglobulin (or fragment thereof) as described

15   herein may contain a detectable moiety or label such as an enzyme, cytotoxic agent or other reporter molecule, including a dye, radionuclide, luminescent group, fluorescent group, or biotin, or the like. The DSP-16-specific immunoglobulin or fragment thereof may be radiolabeled for diagnostic or therapeutic applications. Techniques for radiolabeling of antibodies are known in the art. *See, e.g.*, Adams 1998 *In Vivo* 12:11-

20   21; Hiltunen 1993 *Acta Oncol. 32*:831-9. Therapeutic applications are described in greater detail below and may include use of the DSP-16-binding antibody (or fragment thereof) in conjunction with other therapeutic agents. The antibody or fragment may also be conjugated to a cytotoxic agent as known in the art and provided herein, for example, a toxin, such as a ribosome-inactivating protein, a chemotherapeutic agent, an

25   anti-mitotic agent, an antibiotic or the like.

The invention also contemplates the generation of anti-idiotype antibodies that recognize an antibody (or antigen-binding fragment thereof) that specifically binds to DSP-16 as provided herein, or a variant or fragment thereof. Anti-idiotype antibodies may be generated as polyclonal antibodies or as monoclonal

30   antibodies by the methods described herein, using an anti-DSP-16 antibody (or antigen-binding fragment thereof) as immunogen. Anti-idiotype antibodies or fragments thereof

may also be generated by any of the recombinant genetic engineering methods described above, or by phage display selection. An anti-idiotype antibody may react with the antigen binding site of the anti-DSP-16 antibody such that binding of the anti-DSP-16 antibody to a DSP-16 polypeptide is competitively inhibited. Alternatively, an anti-

5   idiotype antibody as provided herein may not competitively inhibit binding of an anti-DSP-16 antibody to a DSP-16 polypeptide.

As provided herein and according to methodologies well known in the art, polyclonal and monoclonal antibodies may be used for the affinity isolation of DSP-16 polypeptides. *See, e.g.,* Hermanson et al., *Immobilized Affinity Ligand Techniques,*

10   Academic Press, Inc. New York, 1992. Briefly, an antibody (or antigen-binding fragment thereof) may be immobilized on a solid support material, which is then contacted with a sample comprising the polypeptide of interest (*e.g.,* a DSP-16). Following separation from the remainder of the sample, the polypeptide is then released from the immobilized antibody.

15   METHODS FOR DETECTING DSP-16 EXPRESSION

Certain aspects of the present invention provide methods that employ antibodies raised against DSP-16 (or DSP-16 alternate form), or hybridizing polynucleotides, for diagnostic and assay purposes. Certain assays involve using an antibody or other agent to detect the presence or absence of DSP-16 (or DSP-16

20   alternate form), or proteolytic fragments thereof. Alternatively, nucleic acid encoding DSP-16 (or DSP-16 alternate form) may be detected, using standard hybridization and/or PCR techniques. Suitable probes and primers may be designed by those having ordinary skill in the art based on the DSP-16 (or DSP-16 alternate form) cDNA sequence provided herein. Assays may generally be performed using any of a variety of

25   samples obtained from a biological source, such as eukaryotic cells, bacteria, viruses, extracts prepared from such organisms and fluids found within living organisms. Biological samples that may be obtained from a patient include blood samples, biopsy specimens, tissue explants, organ cultures and other tissue or cell preparations. A patient or biological source may be a human or non-human animal, a primary cell

30   culture or culture adapted cell line including but not limited to genetically engineered

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cell lines that may contain chromosomally integrated or episomal recombinant nucleic acid sequences, immortalized or immortalizable cell lines, somatic cell hybrid cell lines, differentiated or differentiatable cell lines, transformed cell lines and the like. In certain preferred embodiments the patient or biological source is a human, and in certain

5 preferred embodiments the biological source is a non-human animal that is a mammal, for example, a rodent (*e.g.,* mouse, rat, hamster, etc.), an ungulate (*e.g.,* bovine) or a non-human primate. In certain other preferred embodiments of the invention, a patient may be suspected of having or being at risk for having a disease associated with altered cellular signal transduction, or may be known to be free of a risk for or presence of such

10 as disease.

To detect DSP-16 (or DSP-16 alternate form) protein, the reagent is typically an antibody, which may be prepared as described below. There are a variety of assay formats known to those having ordinary skill in the art for using an antibody to detect a polypeptide in a sample. *See, e.g.,* Harlow and Lane, *Antibodies: A Laboratory*

15 *Manual,* Cold Spring Harbor Laboratory, 1988. For example, the assay may be performed in a Western blot format, wherein a protein preparation from the biological sample is resolved by gel electrophoresis, transferred to a suitable membrane and allowed to react with the antibody. The presence of the antibody on the membrane may then be detected using a suitable detection reagent, as described below.

20 In another embodiment, the assay involves the use of antibody immobilized on a solid support to bind to the target DSP-16 (or DSP-16 alternate form) and remove it from the remainder of the sample. The bound DSP-16 may then be detected using a second antibody or reagent that contains a reporter group. Alternatively, a competitive assay may be utilized, in which a DSP-16 (or DSP-16

25 alternate form) polypeptide is labeled with a reporter group and allowed to bind to the immobilized antibody after incubation of the antibody with the sample. The extent to which components of the sample inhibit the binding of the labeled polypeptide to the antibody is indicative of the reactivity of the sample with the immobilized antibody, and as a result, indicative of the level of DSP-16 (or DSP-16 alternate form) in the sample.

30 The solid support may be any material known to those having ordinary skill in the art to which the antibody may be attached, such as a test well in a microtiter

plate, a nitrocellulose filter or another suitable membrane. Alternatively, the support may be a bead or disc, such as glass, fiberglass, latex or a plastic such as polystyrene or polyvinylchloride. The antibody may be immobilized on the solid support using a variety of techniques known to those in the art, which are amply described in the patent

5   and scientific literature.

In certain embodiments, the assay for detection of DSP-16 (or DSP-16 alternate form) in a sample is a two-antibody sandwich assay. This assay may be performed by first contacting an antibody that has been immobilized on a solid support, commonly the well of a microtiter plate, with the biological sample, such that DSP-16

10   (or DSP-16 alternate form) within the sample is allowed to bind to the immobilized antibody (a 30 minute incubation time at room temperature is generally sufficient). Unbound sample is then removed from the immobilized DSP-16/antibody complexes and a second antibody (containing a reporter group such as an enzyme, dye, radionuclide, luminescent group, fluorescent group or biotin) capable of binding to a

15   different site on the DSP-16 is added. The amount of second antibody that remains bound to the solid support is then determined using a method appropriate for the specific reporter group. For radioactive groups, scintillation counting or autoradiographic methods are generally appropriate. Spectroscopic methods may be used to detect dyes, luminescent groups and fluorescent groups. Biotin may be detected

20   using avidin, coupled to a different reporter group (commonly a radioactive or fluorescent group or an enzyme). Enzyme reporter groups may generally be detected by the addition of substrate (generally for a specific period of time), followed by spectroscopic or other analysis of the reaction products. Standards and standard additions may be used to determine the level of DSP-16 in a sample, using well known

25   techniques.

In a related aspect of the present invention, kits for detecting DSP-16 and DSP-16 phosphatase activity are provided. Such kits may be designed for detecting the level of DSP-16 or nucleic acid encoding DSP-16, or may detect phosphatase activity of DSP-16 in a direct phosphatase assay or a coupled phosphatase assay. In general, the

30   kits of the present invention comprise one or more containers enclosing elements, such as reagents or buffers, to be used in the assay.

A kit for detecting the level of DSP-16 (or DSP-16 alternate form), or nucleic acid encoding DSP-16 (or DSP-16 alternate form), typically contains a reagent that binds to the DSP-16 protein, DNA or RNA. To detect nucleic acid encoding DSP-16, the reagent may be a nucleic acid probe or a PCR primer. To detect DSP-16 protein,

5   the reagent is typically an antibody. Such kits also contain a reporter group suitable for direct or indirect detection of the reagent (i.e., the reporter group may be covalently bound to the reagent or may be bound to a second molecule, such as Protein A, Protein G, immunoglobulin or lectin, which is itself capable of binding to the reagent). Suitable reporter groups include, but are not limited to, enzymes (e.g., horseradish peroxidase),

10   substrates, cofactors, inhibitors, dyes, radionuclides, luminescent groups, fluorescent groups and biotin. Such reporter groups may be used to directly or indirectly detect binding of the reagent to a sample component using standard methods known to those having ordinary skill in the art.

Kits for detecting DSP-16 activity typically comprise a DSP-16 substrate

15   in combination with a suitable buffer. DSP-16 activity may be specifically detected by performing an immunoprecipation step with a DSP-16-specific antibody prior to performing a phosphatase assay as described above. Other reagents for use in detecting dephosphorylation of substrate may also be provided.

Within certain diagnostic assays, a proliferative disorder may be detected

20   in a patient or any other biological source organism as provided herein based on the presence of an altered DSP-16 (or DSP-16 alternate form) or an altered level of DSP-16 expression. For example, an antibody may distinguish between a wild-type DSP-16 and an altered DSP-16 having a variation in amino acid sequence. Such a variation may be indicative of the presence of a proliferative disorder, or of susceptibility to such a

25   disorder. Hybridization and amplification techniques may be similarly used to detect modified DSP-16 sequences.

METHODS FOR IDENTIFYING MODULATORS OF DSP-16 ACTIVITY

In one aspect of the present invention, DSP-16 (or DSP-16 alternate form) polypeptides may be used to identify agents that modulate DSP-16 activity. Such

30   agents may inhibit or enhance signal transduction via a MAP-kinase cascade, leading to

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cell proliferation. An agent that modulates DSP-16 activity may alter (*e.g.*, increase or decrease in a statistically significant manner) expression and/or stability of DSP-16, DSP-16 protein activity and/or the ability of DSP-16 to dephosphorylate a substrate. Agents that may be screened within such assays include, but are not limited to,

5   antibodies and antigen-binding fragments thereof, competing substrates or peptides that represent, for example, a catalytic site or a dual phosphorylation motif, antisense polynucleotides and ribozymes that interfere with transcription and/or translation of DSP-16 and other natural and synthetic molecules, for example small molecule inhibitors, that bind to and inactivate DSP-16.

10   Candidate agents for use in a method of screening for a modulator of DSP-16 according to the present invention may be provided as "libraries" or collections of compounds, compositions or molecules. Such molecules typically include compounds known in the art as "small molecules" and having molecular weights less than 10^5 daltons, preferably less than 10^4 daltons and still more preferably less than 10^3

15   daltons. For example, members of a library of test compounds can be administered to a plurality of samples, each containing at least one DSP-16 (or DSP-16 alternate form) polypeptide as provided herein, and then assayed for their ability to enhance or inhibit DSP-16-mediated dephosphorylation of, or binding to, a substrate. Compounds so identified as capable of influencing DSP-16 function (*e.g.*, phosphotyrosine and/or

20   phosphoserine/threonine dephosphorylation) are valuable for therapeutic and/or diagnostic purposes, since they permit treatment and/or detection of diseases associated with DSP-16 activity. Such compounds are also valuable in research directed to molecular signaling mechanisms that involve DSP-16, and to refinements in the discovery and development of future DSP-16 compounds exhibiting greater specificity.

25   Candidate agents further may be provided as members of a combinatorial library, which preferably includes synthetic agents prepared according to a plurality of predetermined chemical reactions performed in a plurality of reaction vessels. For example, various starting compounds may be prepared employing one or more of solid-phase synthesis, recorded random mix methodologies and recorded reaction split

30   techniques that permit a given constituent to traceably undergo a plurality of permutations and/or combinations of reaction conditions. The resulting products

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comprise a library that can be screened followed by iterative selection and synthesis procedures, such as a synthetic combinatorial library of peptides (see *e.g.*, PCT/US91/08694, PCT/US91/04666, which are hereby incorporated by reference in their entireties) or other compositions that may include small molecules as provided

5   herein (see *e.g.*, PCT/US94/08542, EP 0774464, U.S. 5,798,035, U.S. 5,789,172, U.S. 5,751,629, which are hereby incorporated by reference in their entireties). Those having ordinary skill in the art will appreciate that a diverse assortment of such libraries may be prepared according to established procedures, and tested using DSP-16 according to the present disclosure.

10   In certain embodiments, modulating agents may be identified by combining a candidate agent with a DSP-16 (or DSP-16 alternate form) polypeptide or a polynucleotide encoding such a polypeptide, *in vitro* or *in vivo*, and evaluating the effect of the candidate agent on the DSP-16 phosphatase activity using, for example, a representative assay described herein. An increase or decrease in phosphatase activity

15   can be measured by performing a representative assay provided herein in the presence and absence of a candidate agent. Briefly, a candidate agent may be included in a mixture of active DSP-16 polypeptide and substrate (*e.g.*, a phosphorylated MAP-kinase), with or without pre-incubation with one or more components of the mixture. In general, a suitable amount of antibody or other agent for use in such an assay ranges

20   from about 0.01 µM to about 100 µM. The effect of the agent on DSP-16 activity may then be evaluated by quantifying the loss of phosphate from the substrate, and comparing the loss with that achieved using DSP-16 without the addition of a candidate agent. Alternatively, a coupled kinase assay may be used, in which DSP-16 activity is indirectly measured based on MAP-kinase activity.

25   Alternatively, a polynucleotide comprising a DSP-16 promoter operably linked to a DSP-16 coding region or reporter gene may be used to evaluate the effect of a test compound on DSP-16 transcription. Such assays may be performed in cells that express DSP-16 endogenously (*e.g.*, human or other mammalian skeletal muscle, heart, brain, liver or pancreatic cells) or in cells transfected with an expression vector

30   comprising a DSP-16 promoter linked to a reporter gene. The effect of a test compound

may then be evaluated by assaying the effect on transcription of DSP-16 or the reporter using, for example, a Northern blot analysis or a suitable reporter activity assay.

DSP-16 activity may also be measured in whole cells transfected with a reporter gene whose expression is dependent upon the activation of an appropriate

5   substrate. For example, appropriate cells (i.e., cells that express DSP-16) may be transfected with a substrate-dependent promoter linked to a reporter gene. In such a system, expression of the reporter gene (which may be readily detected using methods well known to those of ordinary skill in the art) depends upon activation of substrate. Dephosphorylation of substrate may be detected based on a decrease in reporter activity.

10   Candidate modulating agents may be added to such a system, as described above, to evaluate their effect on DSP-16 activity.

The present invention further provides methods for identifying a molecule that interacts with, or binds to, DSP-16 (or DSP-16 alternate form). Such a molecule generally associates with DSP-16 with an affinity constant (K_a) of at least 10^4,

15   preferably at least 10^5, more preferably at least 10^6, still more preferably at least 10^7 and most preferably at least 10^8. Affinity constants may be determined using well known techniques. Methods for identifying interacting molecules may be used, for example, as initial screens for modulating agents, or to identify factors that are involved in the *in vivo* DSP-16 activity. Techniques for substrate trapping, for example using DSP-16

20   variants or substrate trapping mutants as described above, are also contemplated according to certain embodiments provided herein. In addition to standard binding assays, there are many other techniques that are well known for identifying interacting molecules, including yeast two-hybrid screens, phage display and affinity techniques. Such techniques may be performed using routine protocols, which are well known to

25   those having ordinary skill in the art (see, e.g., Bartel et al., In *Cellular Interactions in Development: A Practical Approach*, D.A. Harley, ed., Oxford University Press (Oxford, UK), pp. 153-179, 1993). Within these and other techniques, candidate interacting proteins (e.g., putative DSP-16 substrates) may be phosphorylated prior to assaying for the presence of DSP-16-binding or interacting proteins.

30   Within other aspects, the present invention provides animal models in which an animal either does not express a functional DSP-16 (or DSP-16 alternate

form), or expresses an altered DSP-16. Such animals may be generated using standard homologous recombination strategies. Animal models generated in this manner may be used to study activities of DSP-16 polypeptides and modulating agents *in vivo*.

METHODS FOR DEPHOSPHORYLATING A SUBSTRATE

5   In another aspect of the present invention, a DSP-16 (or DSP-16 alternate form) polypeptide may be used for dephosphorylating a substrate of DSP-16 as provided herein. In one embodiment, a substrate may be dephosphorylated *in vitro* by incubating a DSP-16 polypeptide with a substrate in a suitable buffer (*e.g.*, Tris, pH 7.5, 1 mM EDTA, 1 mM dithiothreitol, 1 mg/mL bovine serum albumin) for 10 minutes at

10   30°C. Any compound that can be dephosphorylated by DSP-16, such as a MAP-kinase, may be used as a substrate. In general, the amounts of the reaction components may range from about 50 pg to about 50 ng of DSP-16 polypeptide and from about 10 ng to about 10 μg of substrate. Dephosphorylated substrate may then be purified, for example, by affinity techniques and/or gel electrophoresis. The extent of substrate

15   dephosphorylation may generally be monitored by adding [γ-^{32}P]labeled substrate to a test aliquot, and evaluating the level of substrate dephosphorylation as described herein.

METHODS FOR MODULATING CELLULAR RESPONSES

        Modulating agents may be used to modulate, modify or otherwise alter (*e.g.*, increase or decrease) cellular responses such as cell proliferation, differentiation

20   and survival, in a variety of contexts, both *in vivo* and *in vitro*. In general, to so modulate (*e.g.*, increase or decrease in a statistically significant manner) such a response, a cell is contacted with an agent that modulates DSP-16 activity, under conditions and for a time sufficient to permit modulation of DSP-16 activity. Agents that modulate a cellular response may function in any of a variety of ways. For

25   example, an agent may modulate a pattern of gene expression (*i.e.*, may enhance or inhibit expression of a family of genes or genes that are expressed in a coordinated fashion). A variety of hybridization and amplification techniques are available for evaluating patterns of gene expression. Alternatively, or in addition, an agent may effect apoptosis or necrosis of the cell, and/or may modulate the functioning of the cell

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cycle within the cell. (See, *e.g.*, Ashkenazi et al., 1998 *Science*, 281:1305; Thornberry et al., 1998 *Science* 281:1312; Evan et al., 1998 *Science* 281:1317; Adams et al., 1998 *Science* 281:1322; and references cited therein.)

Cells treated as described above may exhibit standard characteristics of
5   cells havingaltered proliferation, differentiation or survival properties. In addition, such cells may (but need not) display alterations in other detectable properties, such as contact inhibition of cell growth, anchorage independent growth or altered intercellular adhesion. Such properties may be readily detected using techniques with which those having ordinary skill in the art will be familiar.

10   THERAPEUTIC METHODS

One or more DSP-16 (or DSP-16 alternate form) polypeptides, modulating agents and/or polynucleotides encoding such polypeptides and/or modulating agents may also be used to modulate DSP-16 activity in a patient. As used herein, a "patient" may be any mammal, including a human, and may be afflicted with a
15   condition associated with DSP-16 activity or may be free of detectable disease. Accordingly, the treatment may be of an existing disease or may be prophylactic. Conditions associated with DSP-16 activity include any disorder associated with cell proliferation, including cancer, graft-versus-host disease (GVHD), autoimmune diseases, allergy or other conditions in which immunosuppression may be involved,
20   metabolic diseases, abnormal cell growth or proliferation and cell cycle abnormalities. Certain such disorders involve loss of normal MAP-kinase phosphatase activity, leading to uncontrolled cell growth. DSP-16 polypeptides, and polynucleotides encoding such polypeptides, can be used to ameliorate such disorders. Activators of DSP-16 may also be used to treat certain disorders, including Duchenne Muscular Dystrophy.

25   For administration to a patient, one or more polypeptides, polynucleotides and/or modulating agents are generally formulated as a pharmaceutical composition. A pharmaceutical composition may be a sterile aqueous or non-aqueous solution, suspension or emulsion, which additionally comprises a physiologically acceptable carrier (*i.e.*, a non-toxic material that does not interfere with the activity of
30   the active ingredient). Such compositions may be in the form of a solid, liquid or gas

(aerosol). Alternatively, compositions of the present invention may be formulated as a lyophilizate or compounds may be encapsulated within liposomes using well known technology. Pharmaceutical compositions within the scope of the present invention may also contain other components, which may be biologically active or inactive. Such

5   components include, but are not limited to, buffers (e.g., neutral buffered saline or phosphate buffered saline), carbohydrates (e.g., glucose, mannose, sucrose or dextrans), mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, chelating agents such as EDTA or glutathione, stabilizers, dyes, flavoring agents, and suspending agents and/or preservatives.

10   Any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of the present invention. Carriers for therapeutic use are well known, and are described, for example, in Remingtons Pharmaceutical Sciences, Mack Publishing Co. (A.R. Gennaro ed. 1985). In general, the type of carrier is selected based on the mode of administration. Pharmaceutical

15   compositions may be formulated for any appropriate manner of administration, including, for example, topical, oral, nasal, intrathecal, rectal, vaginal, sublingual or parenteral administration, including subcutaneous, intravenous, intramuscular, intrasternal, intracavernous, intrameatal or intraurethral injection or infusion. For parenteral administration, the carrier preferably comprises water, saline, alcohol, a fat, a

20   wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, kaolin, glycerin, starch dextrins, sodium alginate, carboxymethylcellulose, ethyl cellulose, glucose, sucrose and/or magnesium carbonate, may be employed.

A pharmaceutical composition (e.g., for oral administration or delivery

25   by injection) may be in the form of a liquid (e.g., an elixir, syrup, solution, emulsion or suspension). A liquid pharmaceutical composition may include, for example, one or more of the following: sterile diluents such as water for injection, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono or diglycerides which may serve as the solvent or suspending

30   medium, polyethylene glycols, glycerin, propylene glycol or other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or

sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. A parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. The use of

5   physiological saline is preferred, and an injectable pharmaceutical composition is preferably sterile.

The compositions described herein may be formulated for sustained release (i.e., a formulation such as a capsule or sponge that effects a slow release of compound following administration). Such compositions may generally be prepared

10   using well known technology and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Sustained-release formulations may contain an agent dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate controlling membrane. Carriers for use within such formulations are biocompatible, and may also be biodegradable; preferably the

15   formulation provides a relatively constant level of active component release. The amount of active compound contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

For pharmaceutical compositions comprising a polynucleotide encoding

20   a DSP-16 polypeptide and/or modulating agent (such that the polypeptide and/or modulating agent is generated in situ), the polynucleotide may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid, and bacterial, viral and mammalian expression systems. Techniques for incorporating DNA into such expression systems are well known to those of ordinary

25   skill in the art. The DNA may also be "naked," as described, for example, in Ulmer et al., Science 259:1745-1749, 1993 and reviewed by Cohen, Science 259:1691-1692, 1993. The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells.

Within a pharmaceutical composition, a DSP-16 (or DSP-16 alternate

30   form) polypeptide, polynucleotide or modulating agent may be linked to any of a variety of compounds. For example, such an agent may be linked to a targeting moiety (e.g., a

monoclonal or polyclonal antibody, a protein or a liposome) that facilitates the delivery of the agent to the target site. As used herein, a "targeting moiety" may be any substance (such as a compound or cell) that, when linked to an agent enhances the transport of the agent to a target cell or tissue, thereby increasing the local concentration

5   of the agent. Targeting moieties include antibodies or fragments thereof, receptors, ligands and other molecules that bind to cells of, or in the vicinity of, the target tissue. An antibody targeting agent may be an intact (whole) molecule, a fragment thereof, or a functional equivalent thereof. Examples of antibody fragments are $F(ab')_2$, -Fab', Fab and F[v] fragments, which may be produced by conventional methods or by genetic or

10   protein engineering. Linkage is generally covalent and may be achieved by, for example, direct condensation or other reactions, or by way of bi- or multi-functional linkers. Targeting moieties may be selected based on the cell(s) or tissue(s) toward which the agent is expected to exert a therapeutic benefit.

Pharmaceutical compositions may be administered in a manner

15   appropriate to the disease to be treated (or prevented). An appropriate dosage and a suitable duration and frequency of administration will be determined by such factors as the condition of the patient, the type and severity of the patient's disease, the particular form of the active ingredient and the method of administration. In general, an appropriate dosage and treatment regimen provides the agent(s) in an amount sufficient

20   to provide therapeutic and/or prophylactic benefit (e.g., an improved clinical outcome, such as more frequent complete or partial remissions, or longer disease-free and/or overall survival). For prophylactic use, a dose should be sufficient to prevent, delay the onset of or diminish the severity of a disease associated with cell proliferation.

Optimal dosages may generally be determined using experimental

25   models and/or clinical trials. In general, the amount of polypeptide present in a dose, or produced in situ by DNA present in a dose, ranges from about 0.01 μg to about 100 μg per kg of host, typically from about 0.1 μg to about 10 μg. The use of the minimum dosage that is sufficient to provide effective therapy is usually preferred. Patients may generally be monitored for therapeutic or prophylactic effectiveness using assays

30   suitable for the condition being treated or prevented, which will be familiar to those

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having ordinary skill in the art.　　Suitable dose sizes will vary with the size of the patient, but will typically range from about 10 mL to about 500 mL for 10-60 kg animal.

The following Example is offered by way of illustration and not by way of limitation.

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EXAMPLES

EXAMPLE 1

CLONING AND SEQUENCING cDNA ENCODING DSP-16

This Example illustrates the cloning of a cDNA molecule encoding human DSP-16.

A conserved sequence motif surrounding the active site domain of dual-specificity phosphatases was identified as follows: Dual specificity phosphatases belong to the larger family of protein tyrosine phosphatases (PTPs) that share a conserved catalytic domain containing a cysteine residue situated N-terminal to a stretch of five variable amino acids followed by an arginine residue (Fauman et al., *Trends In Bioch. Sci.* 21:413-417, 1996). DSPs typically contain a PTP active site motif but lack sequence homology to PTPs in other regions (Jia, *Biochem. and Cell Biol.* 75:17-26, 1997). There is, however, no reported consensus sequence that is conserved among DSPs, nor is a consensus region apparent from examination of the known DSP sequences such as those referred to above.

To derive a longer consensus DSP amino acid sequence motif that would be useful for the identification of new DSP family members, multiple known human dual-specificity phosphatases sequences were aligned and compared. An alignment of eight amino acid sequences derived from eight human DSPs having MAP-kinase phosphatase activity yielded a conserved homology region consisting of a 24-amino acid peptide sequence containing the PTP active site signature motif. Thus, a candidate peptide having the sequence:

NGRVLVHCQAGISRSGTNILAYLM   SEQ ID NO:17

was used to search the "month" database (Nat. Center for Biol. Information, www.ncbi.nlm.nih.gov/blast.cgi?Jform=1). The search employed an algorithm (tblastn) capable of reverse translation of the candidate peptide with iterations allowing for genetic code degeneracy within default parameters.

The search results identified the EST AC007619 in the human genomic sequence database. Deduced translated sequences of AC007619 were analyzed to determine an open reading frame encoding a PTP active site domain, revealing the presence within this EST of a nucleotide region encoding a 50 amino acid polypeptide

5   containing such a site, in a reading frame that was immediately downstream of three successive stop codons and immediately upstream of a stop codon. The amino acid sequence containing the C-terminal 41 amino acids of this peptide was as follows:

KAKASNGCVLVHCLAGISRSATIAIAYIMKRMDMSLDEAYR   SEQ ID NO: 13

10

When this 41 amino acid peptide sequence encoded by AC007619 was resubmitted to a BLAST (tblastn) search of the GenBank EST database, a bovine EST, AW461438, was identified that was capable of encoding a polypeptide containing the same PTP catalytic domain sequence as was present in the 41 amino acid query sequence.

15   Resubmission of AW461438 as a query sequence to the GenBank "nr" (nonredundant) database revealed alignment of the query sequence with the putative exon regions of AC007619. The alignment of AC007619 with AW461438 was then used to derive portions of AC007619 comprising a theoretical human DSP cDNA sequence, by aligning regions of AW461438 with three presumptive exon regions of

20 AC007619 and identifying classical splice junction (donor/acceptor) sites in AC007619. AW461438 exhibited greater than 90% homology with the exon regions of AC007619 by this alignment. This theoretical human DSP cDNA sequence was then used as a blastn query in the GenBank "dbest" database (www.ncbi.nlm.nih.gov/dbest). The human EST AW847425 was identified by this search; the 3' end of the reverse

25 complement of AW847425 contained a 130 nucleotide overlap with the theoretical human DSP cDNA sequence. AW847425 also contained additional putative exon regions that were present in AC007619. A concensus sequence was deduced from the alignment of AW847425 with the theoretical human DSP cDNA sequence identified as described above (i.e., by alignment of AC007619 and AW461438). Analysis of the

30 deduced consensus sequence showed the presence of six exons with only three nucleotides mismatched. Where there was a mismatch, the nucleotide situated at the

corresponding position in AC007619 was used to arrive at the consensus sequence. From this concensus sequence, the following PCR primers were designed:

PCR—5':

5   CAA AGT GTT AAT TAC AGA GCT CAT CCA GCA TTC AGC GA—3'

SEQ ID NO: 14

PCR—3':

5'—TTG GCT TCT CCA GGT GCA GCA GCT TGA GTT—3'   SEQ ID NO: 15

10

PCR reactions were conducted under standard conditions with these primers using as a template thymus cDNA made by reverse transcription of human thymus total RNA (Clontech, Palo Alto, CA) total RNA as template. PCR products were sequenced on a GeneAnalyzer™ DNA sequencer (Perkin-Elmer Corp., Applied

15   Biosystems Division, Foster City, CA) according to the manufacturer's instructions. The sequences of the PCR products confirmed the existence of an actual polynucleotide having the base sequence identified as the consensus sequence by alignments of database members, as described above. The consensus nucleotide sequence was used to design 5' and 3' RACE primers as follows:

20

GSP5':

5'—CAT TTC CTG CGT GTG CCT GTG AAT GAC AGC TT—3'   SEQ ID NO: 18

GSP2':

25   5'—AAG AGA GAG AGG CAA CAT CTT GGG AGC TTT GAT CG—3'

SEQ ID NO: 19

5' and 3' RACE (rapid amplification of cDNA ends) analysis (Frohman et al., *Proc. Nat. Acad. Sci. USA* 85:8998, 1988; Ohara et al., *Proc. Nat. Acad. Sci. USA*

30   86:5673, 1989; Loh et al., *Science* 243:217, 1989) was performed using either thymus (5') or brain, testis and skeletal muscle (3') cDNA templates with 5'/3' RACE kits

(Roche Molecular Biochemicals, Inc. (formerly Boehringer Mannheim), Indianapolis, IN; Clontech, Palo Alto, CA; Life Technologies, Gaithersburg, MD) according to the suppliers' instructions, and the reaction products were sequenced according to standard procedures. A cDNA of 3496 nucleotides was identified including the 1995 base DSP-

5   16 encoding nucleotide sequence, as shown in Figure 1 (SEQ ID NO:1) and encoding a 665 amino acid DSP-16 polypeptide as shown in Figure 2 (SEQ ID NO:2).

An additional product of the PCR reaction described above was a second cDNA of 3332 bases (Figure 3, SEQ ID NO:20) including a 1551 nucleotide DSP-16 alternate form-encoding sequence, as shown in Figure 3 and encoding a protein of 517

10   amino acids (Figure 4, SEQ ID NO:21). Apparently (and according to non-limiting theory) this cDNA lacks one exonic sequence relative to the DSP-16 coding sequence of SEQ ID NO:1 and thus encodes a truncated DSP-16 produced by alternate splicing of a DSP-16 encoding transcipt.

As shown in Figure 5, DSP-16 (and DSP-16 alternate form) exhibit high

15   homology with other MAP kinase phosphatases, in particular, hVH5. Although this polypeptide sequence alignment of DSP-16 with hVH5 indicated a strong similarity at the amino acid sequence level (alignment score of 44% with CLUSTALW, v. 1.73), there was significantly lower similarity (37%) between the coding sequences for these two proteins when the nucleotide sequences were analyzed using CLUSTALW. The

20   identified cDNA contained the 1995 base pair coding region, as well as associated 5' and 3' untranslated sequences. Based on the presence of human chromosome 12p sequences in the database entry AC007619, which shares exon encoding sequences with DSP-16 as described above, the chromosomal location of the gene encoding DSP-16 was assigned to human chromosome 12p.

25

EXAMPLE 2

DSP-16 EXPRESSION IN HUMAN TISSUES

In this example, a DSP-16 encoding nucleic acid sequence is shown to hybridize to human polyA+ RNA from various tissue sources. Full length DSP-16

5 encoding cDNA (SEQ ID NO:1) is ^{32}P-labeled by the random primer method as described in Ausubel et al. (1998 *Current Protocols in Molecular Biology*, Greene Publ. Assoc. Inc. & John Wiley & Sons, Inc., Boston, MA) for use as a nucleic acid hybridization probe. The probe is hybridized to blots containing human polyA+ RNA derived from multiple human tissues, normalized for the amount of detectable β-actin

10 mRNA (Cat. No. 7759-1; Clontech, Inc., Palo Alto, CA). Blots undergo prehybridization for 30 min at 68°C in Express Hyb™ solution (Clontech), and then are hybridized with the labeled probe for 1 hour at 68°C in Express Hyb™ solution. The blots are next washed for 40 min at room temperature in 2X SSC, 0.05% SDS, followed by a second wash for 40 min at 50°C in 0.1X SSC, 0.1% SDS. Blots are exposed to

15 Hyperfilm MP™ autoradiographic film (Amersham Life Sciences, Arlington Hts, IL) overnight. Results are shown in Figure 4, in which the human tissue sources for the RNAs are as follows: Lane 1, heart; lane 2, brain; lane 3, placenta; lane 4, lung; lane 5, liver; lane 6, skeletal muscle; lane 7, kidney; lane 8, pancreas.

20 EXAMPLE 3

DSP-16 PHOSPHATASE ACTIVITY

Assays of DSP-16 activity using a tyrosine phosphorylated ^{32}P-labeled EGF receptor autophosphorylation site peptide as substrate are performed essentially as described (Flint et al., 1993 *EMBO J. 12*:1937-1946; Zhang et al., 1994 *Biochem.*

25 *33*:2285-2290). A polynucleotide comprising the DSP-16 coding sequence of SEQ ID NO:1 is cloned into the pGEX expression vector (Pharmacia, Piscataway, NJ) and expressed in *E. coli* as a DSP-16-glutathione-S-transferase (GST) fusion protein according to the supplier's instructions. Affinity isolation of the DSP-16-GST fusion protein on immobilized glutathione (Pharmacia) following extraction is also conducted

30 as recommended by the supplier. All reagents are from Sigma Chemical Co. (St. Louis,

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MO) unless otherwise noted. An aliquot (20 µl) of ice-cold Assay Buffer (25 mM imidazole (EM Science, Gibbstown, NJ)-pH 7.2, 1 mM EDTA, 2 mM dithiothreitol (DTT, Roche Molecular Biochemicals, Indianapolis, IN), 0.25 mg/ml ovalbumin (Calbiochem-Novabiochem, La Jolla, CA)) is added to wells designated as enzyme

5   negative controls. DSP-16 (SEQ ID NO:2) diluted into ice-cold Assay Buffer from a 50% glycerol stock such that this amount of enzyme would utilize less than 20% of the substrate in the assay, is added, 20 µl per well to all wells except enzyme negative control wells. The plate is agitated for 20 sec to mix the contents of each well and incubated for 13 min at room temperature. For substrate, the autophosphorylation site

10   from the EGF receptor having the amino acid sequence DADEpYL-NH$_2$ [SEQ ID NO: 22] is prepared as a ^{32}P-labeled substrate peptide essentially as described (Zhang et al., 1994 Biochem. 33:2285; specific activity 11 µCi/nMol), diluted to 0.6 µM in Assay Buffer, and added to all wells in 20 µl aliquots. The plate is again agitated and then incubated an additional 13 minutes, at which time 140 µl of an activated charcoal

15   suspension (25 mg/ml in 0.1 M NaH$_2$PO$_4$, pH$\leq$5) is added to each well, the contents mixed by vortexing, and the plate is then centrifuged 2400 rpm for three min at room temperature in a tabletop centrifuge (Beckman Instruments, Inc., Fullerton, CA). Aliquots (100 µl) of the supernatant fluid in each well are transferred to a beta-scintillation counting plate (Wallac, Inc., Gaithersburg, MD) and ^{32}P beta emissions are

20   quantified using a Wallac Microbeta™ plate counter according to the manufacturer's recommendations. After subtracting background counts, correcting for enzyme negative control values and normalizing to control wells DSP-16 specific activity for the EGF receptor peptide substrate is calculated toand expressed as nmole/min/mg, and a Km value is determined.

25

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for the purpose of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the present invention is not limited except as by the appended

30   claims.

CLAIMS

1.   An isolated polypeptide having the sequence of DSP-16 recited in SEQ ID NO:2, or a variant thereof that differs in one or more amino acid deletions, additions, insertions or substitutions at no more than 50% of the residues in SEQ ID NO:2, such that the polypeptide retains the ability to dephosphorylate an activated MAP-kinase.

2.   An isolated polynucleotide that encodes at least ten consecutive amino acids of a polypeptide having a sequence corresponding to SEQ ID NO:2.

3.   An isolated polynucleotide that encodes at least fifteen consecutive amino acids of a polypeptide having a sequence corresponding to SEQ ID NO:2.

4.   An expression vector comprising a polynucleotide according to claim 2 or 3.

5.   A host cell transformed or transfected with an expression vector according to claim 4.

6.   An isolated polynucleotide that encodes a polypeptide according to claim 1.

7.   A polynucleotide according to claim 6, comprising the sequence recited in SEQ ID NO:1.

8.   An expression vector comprising a polynucleotide according to claim 6.

9.   A host cell transformed or transfected with an expression vector according to claim 8.

10.   An antisense polynucleotide comprising at least 15 consecutive nucleotides complementary to a polynucleotide according to claim 6.

11.   An isolated polynucleotide that detectably hybridizes to the complement of the sequence recited in SEQ ID NO:1 under conditions that include a wash in 0.1X SSC and 0.1% SDS at 50 °C for 15 minutes.

12.   An expression vector comprising a polynucleotide according to claim 10 or claim 11.

13.   A host cell transformed or transfected with an expression vector according to claim 12.

14.   A method of producing a DSP-16 polypeptide, comprising the steps of:

    (a)   culturing a host cell according to claim 9 under conditions that permit expression of the DSP-16 polypeptide; and

    (b)   isolating DSP-16 polypeptide from the host cell culture.

15.   An isolated antibody, or antigen binding fragment thereof, that specifically binds to a DSP-16 polypeptide having the sequence of SEQ ID NO:2.

16.   An antibody or fragment thereof according to claim 15, wherein the antibody is a monoclonal antibody.

17.   A pharmaceutical composition comprising an antibody or fragment thereof according to claim 15 in combination with a physiologically acceptable carrier.

18.   A method for detecting DSP-16 expression in a sample, comprising:

(a)   contacting a sample with an antibody or an antigen-binding fragment thereof according to claim 15, under conditions and for a time sufficient to allow formation of an antibody/DSP-16 complex; and

(b)   detecting the level of antibody/DSP-16 complex, and therefrom detecting the presence of DSP-16 in a sample.

19.   A method according to claim 18, wherein the antibody is linked to a support material.

20.   A method according to claim 18, wherein the antibody is linked to a detectable marker.

21.   A method according to claim 18, wherein the sample is a biological sample obtained from a patient.

22.   A method for detecting DSP-16 expression in a sample, comprising:

(a)   contacting a sample with an antisense polynucleotide according to claim 10 or claim 11; and

(b)   detecting in the sample an amount of DSP-16 polynucleotide that hybridizes to the antisense polynucleotide, and therefrom detecting DSP-16 expression in the sample.

23.   A method according to claim 22, wherein the amount of DSP-16 polynucleotide that hybridizes to the antisense polynucleotide is determined using polymerase chain reaction.

24.   A method according to claim 22, wherein the amount of DSP-16 polynucleotide that hybridizes to the antisense polynucleotide is determined using a hybridization assay.

25.   A method according to claim 22, wherein the sample comprises an RNA or cDNA preparation.

26.   A method for screening for an agent that modulates DSP-16 activity, comprising the steps of:

(a)   contacting a candidate agent with a polypeptide according to claim 1, under conditions and for a time sufficient to permit interaction between the polypeptide and candidate agent; and

(b)   subsequently evaluating the ability of the polypeptide to dephosphorylate a DSP-16 substrate, relative to a predetermined ability of the polypeptide to dephosphorylate the DSP-16 substrate in the absence of candidate agent; and therefrom identifying an agent that modulates DSP-16 activity.

27.   A method according to claim 26, wherein the DSP-16 substrate is a MAP-kinase.

28.   A method according to claim 26, wherein the candidate agent is a small molecule.

29.   A method according to claim 26, wherein the small molecule is present within a combinatorial library.

30.   A method for screening for an agent that modulates DSP-16 activity, comprising the steps of:

(a)   contacting a candidate agent with a cell comprising a DSP-16 promoter operably linked to a polynucleotide encoding a detectable transcript or protein,

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under conditions and for a time sufficient to permit interaction between the promoter and candidate agent; and

(b)   subsequently evaluating the expression of the polynucleotide, relative to a predetermined level of expression in the absence of candidate agent;

and therefrom identifying an agent that modulates DSP-16 activity.

31.   A method according to claim 30, wherein the polynucleotide encodes a DSP-16 polypeptide.

32.   A method according to claim 30, wherein the polynucleotide encodes a reporter protein.

33.   A method for modulating a proliferative response in a cell, comprising contacting a cell with an agent that modulates DSP-16 activity.

34.   A method for modulating differentiation of a cell, comprising contacting a cell with an agent that modulates DSP-16 activity.

35.   A method for modulating survival of a cell, comprising contacting a cell with an agent that modulates DSP-16 activity.

36.   A method according to any one of claims 33-35, wherein the agent modulates a pattern of gene expression.

37.   A method according to any one of claims 33-35, wherein the cell displays contact inhibition of cell growth.

38.   A method according to any one of claims 33-35, wherein the cell displays anchorage independent growth.

39.   A method according to any one of claims 33-35, wherein the cell displays an altered intercellular adhesion property.

40.   A method according to claim 35, wherein the agent modulates apoptosis.

41.   A method according to claim 35, wherein the agent modulates the cell cycle.

42.   A method according to claim 32, wherein the cell is present within a patient.

43.   A method for treating a patient afflicted with a disorder associated with DSP-16 activity, comprising administering to a patient a therapeutically effective amount of an agent that modulates DSP-16 activity.

44.   A method according to claim 43, wherein the disorder is selected from the group consisting of Duchenne muscular dystrophy, cancer, graft-versus-host disease, autoimmune diseases, allergies, metabolic diseases, abnormal cell growth, abnormal cell proliferation and cell cycle abnormalities.

45.   A DSP-16 substrate trapping mutant polypeptide that differs from the sequence recited in SEQ ID NO:2 in one or more amino acid deletions, additions, insertions or substitutions at no more than 50% of the residues in SEQ ID NO:2, such that the polypeptide binds to a substrate with an affinity that is not substantially diminished relative to DSP-16, and such that the ability of the polypeptide to dephosphorylate a substrate is reduced relative to DSP-16.

46.   A substrate trapping mutant polypeptide according to claim 45, wherein the polypeptide contains a substitution at position 213 or position 244 of SEQ ID NO:2.

47.   A method for screening a molecule for the ability to interact with DSP-16, comprising the steps of:

(a)   contacting a candidate molecule with a polypeptide according to claim 1 under conditions and for a time sufficient to permit the candidate molecule and polypeptide to interact; and

(b)   detecting the presence or absence of binding of the candidate molecule to the polypeptide, and therefrom determining whether the candidate molecule interacts with DSP-16.

48.   A method according to claim 47, wherein the step of detecting comprises an affinity purification step.

49.   A method according to claim 47, wherein the step of detecting comprises a yeast two hybrid screen or a screen of a phage display library.

50.   An isolated polypeptide comprising the sequence of DSP-16 alternate form recited in SEQ ID NO:21, or a variant thereof that differs in one or more amino acid deletions, additions, insertions or substitutions at no more than 50% of the residues in SEQ ID NO:21, such that the polypeptide retains the ability to dephosphorylate an activated MAP-kinase.

51.   An isolated polynucleotide that encodes at least ten consecutive amino acids of a polypeptide having a sequence corresponding to SEQ ID NO:21.

52.   An isolated polynucleotide that encodes at least fifteen consecutive amino acids of a polypeptide having a sequence corresponding to SEQ ID NO:21.

53.   An expression vector comprising a polynucleotide according to claim 51 or 52.

54.   A host cell transformed or transfected with an expression vector according to claim 53.

55.   An isolated polynucleotide that encodes a polypeptide according to claim 50.

56.   A polynucleotide according to claim 55, comprising the sequence recited in SEQ ID NO:20.

57.   An expression vector comprising a polynucleotide according to claim 55.

58.   A host cell transformed or transfected with an expression vector according to claim 57.

59.   An antisense polynucleotide comprising at least 15 consecutive nucleotides complementary to a polynucleotide according to claim 55.

60.   An isolated polynucleotide that detectably hybridizes to the complement of the sequence recited in SEQ ID NO:20 under conditions that include a wash in 0.1X SSC and 0.1% SDS at 60 °C for 15 minutes.

61.   An expression vector comprising a polynucleotide according to claim 59 or claim 60.

62.   A host cell transformed or transfected with an expression vector according to claim 61.

63.   A method of producing a DSP-16 alternate form polypeptide, comprising the steps of:

(a)   culturing a host cell according to claim 58 under conditions that permit expression of the DSP-16 alternate form polypeptide; and

(b)   isolating DSP-16 alternate form polypeptide from the host cell culture.

64.   An isolated antibody, or antigen binding fragment thereof, that specifically binds to a DSP-16 alternate form polypeptide having the sequence of SEQ ID NO:21.

65..   An antibody or fragment thereof according to claim 64, wherein the antibody is a monoclonal antibody.

66.   A pharmaceutical composition comprising an antibody or fragment thereof according to claim 64 in combination with a physiologically acceptable carrier.

67.   A method for detecting DSP-16 alternate form expression in a sample, comprising:

(a)   contacting a sample with an antibody or an antigen-binding fragment thereof according to claim 64, under conditions and for a time sufficient to allow formation of an antibody/DSP-16 alternate form complex; and

(b)   detecting the level of antibody/DSP-16 alternate form complex, and therefrom detecting the presence of DSP-16 alternate form in a sample.

68.   A method according to claim 67, wherein the antibody is linked to a support material.

69.   A method according to claim 67, wherein the antibody is linked to a detectable marker.

70.   A method according to claim 67, wherein the sample is a biological sample obtained from a patient.

71.   A method for detecting DSP-16 alternate form expression in a sample, comprising:

(a)   contacting a sample with an antisense polynucleotide according to claim 59 or claim 60; and

(b)   detecting in the sample an amount of DSP-16 alternate form polynucleotide that hybridizes to the antisense polynucleotide, and therefrom detecting DSP-16 alternate form expression in the sample.

72.   A method according to claim 71, wherein the amount of DSP-16 alternate form polynucleotide that hybridizes to the antisense polynucleotide is determined using polymerase chain reaction.

73.   A method according to claim 71, wherein the amount of DSP-16 alternate form polynucleotide that hybridizes to the antisense polynucleotide is determined using a hybridization assay.

74.   A method according to claim 71, wherein the sample comprises an RNA or cDNA preparation.

75.   A method for screening for an agent that modulates DSP-16 alternate form activity, comprising the steps of:

(a)   contacting a candidate agent with a polypeptide according to claim 50, under conditions and for a time sufficient to permit interaction between the polypeptide and candidate agent; and

(b)   subsequently evaluating the ability of the polypeptide to dephosphorylate a DSP-16 alternate form substrate, relative to a predetermined ability of the polypeptide to dephosphorylate the DSP-16 alternate form substrate in the absence of candidate agent;

and therefrom identifying an agent that modulates DSP-16 alternate form activity.

76.   A method according to claim 75, wherein the DSP-16 alternate form substrate is a MAP-kinase.

77.   A method according to claim 75, wherein the candidate agent is a small molecule.

78.   A method according to claim 75, wherein the small molecule is present within a combinatorial library.

79.   A method for screening for an agent that modulates DSP-16 alternate form activity, comprising the steps of:

(a)   contacting a candidate agent with a cell comprising a DSP-16 alternate form promoter operably linked to a polynucleotide encoding a detectable transcript or protein, under conditions and for a time sufficient to permit interaction between the promoter and candidate agent; and

(b)   subsequently evaluating the expression of the polynucleotide, relative to a predetermined level of expression in the absence of candidate agent;

and therefrom identifying an agent that modulates DSP-16 alternate form activity.

80.   A method according to claim 79, wherein the polynucleotide encodes a DSP-16 alternate form polypeptide.

81.   A method according to claim 79, wherein the polynucleotide encodes a reporter protein.

82.   A method for modulating a proliferative response in a cell, comprising contacting a cell with an agent that modulates DSP-16 alternate form activity.

83.   A method for modulating differentiation of a cell, comprising contacting a cell with an agent that modulates DSP-16 alternate form activity.

84.   A method for modulating survival of a cell, comprising contacting a cell with an agent that modulates DSP-16 alternate form activity.

85.   A method according to any one of claims 82-84, wherein the agent modulates a pattern of gene expression.

86.   A method according to any one of claims 82-84, wherein the cell displays contact inhibition of cell growth.

87.   A method according to any one of claims 82-84, wherein the cell displays anchorage independent growth.

88.   A method according to any one of claims 82-84, wherein the cell displays an altered intercellular adhesion property.

89.   A method according to claim 84, wherein the agent modulates apoptosis.

90.   A method according to claim 84, wherein the agent modulates the cell cycle.

91.   A method according to claim 81, wherein the cell is present within a patient.

92.   A method for treating a patient afflicted with a disorder associated with DSP-16 alternate form activity, comprising administering to a patient a therapeutically effective amount of an agent that modulates DSP-16 alternate form activity.

93.   A method according to claim 92, wherein the disorder is selected from the group consisting of cancer, graft-versus-host disease, autoimmune diseases, allergies, metabolic diseases, abnormal cell growth, abnormal cell proliferation and cell cycle abnormalities.

94.   A DSP-16 alternate form substrate trapping mutant polypeptide that differs from the sequence recited in SEQ ID NO:21 in one or more amino acid deletions, additions, insertions or substitutions at no more than 50% of the residues in SEQ ID NO:21, such that the polypeptide binds to a substrate with an affinity that is not substantially diminished relative to DSP-16 alternate form, and such that the ability of the polypeptide to dephosphorylate a substrate is reduced relative to DSP-16 alternate form.

95.   A substrate trapping mutant polypeptide according to claim 94, wherein the polypeptide contains a substitution at position 213 or position 244 of SEQ ID NO:21.

96.   A method for screening a molecule for the ability to interact with DSP-16 alternate form, comprising the steps of:

(a)   contacting a candidate molecule with a polypeptide according to claim 50 under conditions and for a time sufficient to permit the candidate molecule and polypeptide to interact; and

(b)   detecting the presence or absence of binding of the candidate molecule to the polypeptide, and therefrom determining whether the candidate molecule interacts with DSP-16 alternate form.

97.   A method according to claim 96, wherein the step of tir comprises an affinity purification step.

98.   A method according to claim 96, wherein the step of tir comprises a yeast two hybrid screen or a screen of a phage display library.

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GAGAGAAGGA GAAGATAATA TACTGAAAAG AAGAGGAGGA GGAGAGCGAC GGGACGGGAC 60
GCGAGCGGGA GCGCAGCCGC CCTCTCGGCT CCGCGGCGGC GCCTCGCAAG TCCGGGAGGC 120
GAGGGGGGCC CGAGGGGAGA CGCCGTGACA ACTTTCGTTT CCCTCTGAGG GAATTGGGAG 180
GTCGGCGGCC CCAAAAGCTT TCAGTCCAGT GTAAAGCTGT TGGAGCGCGG GAGCAAAGGT 240
AAAGAATGAT GTAATGCGCT GGCTGCTCCA AAGCATCTTT TGTTGTGGAA TGGTTATTCC 300
AGTCATCTCT TTATGAATCA AATGTGAGGG GCTGCTTTGT GGACGGAGTC CTTTGCAAGA 360
GCACATCAAC GGGAAAGAGA AAGAGACATT CACTTGGAGG GCTCTTGCTG AAAATGGGTT 420
TAACTCTCCT TTTGCCAGTC ACCACCAGCC TGACCTCATA CACTTTTAGT ACAATGGAGT 480
GGCTGAGCCT TTGAGCACAC CACCATTACA TCATCGTGGC AAATTAAAGA AGGAGGTGGG 540
AAAAGAGGAC TTATTGTTGT CATGGCCCAT GAGATGATTG GAACTCAAAT TGTTACTGAG 600
AGGTTGGTGG CTCTGCTGGA AAGTGGAACG GAAAAAGTGC TGCTAATTGA TAGCCGGCCA 660
TTTGTGGAAT ACAATACATC CCACATTTTG GAAGCCATTA ATATCAACTG CTCCAAGCTT 720
ATGAAGCGAA GGTTGCAACA GGACAAAGTG TTAATTACAG AGCTCATCCA GCATTCAGCG 780
AAACATAAGG TTGACATTGA TTGCAGTCAG AAGGTTGTAG TTTACGATCA AAGCTCCCAA 840
GATGTTGCCT CTCTCTCTTC AGACTGTTTT CTCACTGTAC TTCTGGGTAA ACTGGAGAAG 900
AGCTTCAACT CTGTTCACCT GCTTGCAGGT GGGTTTGCTG AGTTCTCTCG TTGTTTCCCT 960
GGCCTCTGTG AAGGAAAATC CACTCTAGTC CCTACCTGCA TTTCTCAGCC TTGCTTACCT 1020
GTTGCCAACA TTGGGCCAAC CCGAATTCTT CCCAATCTTT ATCTTGGCTG CCAGCGAGAT 1080
GTCCTCAACA AGGAGCTGAT GCAGCAGAAT GGGATTGGTT ATGTGTTAAA TGCCAGCAAT 1140
ACCTGTCCAA AGCCTGACTT TATCCCCGAG TCTCATTTCC TGCGTGTGCC TGTGAATGAC 1200
AGCTTTTGTG AGAAAATTTT GCCGTGGTTG GACAAATCAG TAGATTTCAT TGAGAAAGCA 1260
AAAGCCTCCA ATGGATGTGT TCTAGTGCAC TGTTTAGCTG GGATCTCCCG CTCCGCCACC 1320
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GTGAAAGAAA AAAGACCTAC TATATCTCCA AACTTCAATT TTCTGGGCCA ACTCCTGGAC 1440
TATGAGAAGA AGATTAAGAA CCAGACTGGA GCATCAGGGC CAAAGAGCAA ACTCAAGCTG 1500
CTGCACCTGG AGAAGCCAAA TGAACCTGTC CCTGCTGTCT CAGAGGGTGG ACAGAAAAGC 1560
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AGCCCGCTGG TACAGGCGCT CAGTGGGCTG CACCTGTCCG CAGACAGGCT GGAAGACAGC 1740
AATAAGCTCA AGCGTTCCTT CTCTCTGGAT ATCAAATCAG TTTCATATTC AGCCAGCATG 1800
GCAGCATCCT TACATGGCTT CTCCTCATCA GAAGATGCTT TGGAATACTA CAAACCTTCC 1860
ACTACTCTGG ATGGGACCAA CAAGCTATGC CAGTTCTCCC CTGTTCAGGA ACTATCGGAG 1920
CAGACTCCCG AAACCAGTCC TGATAAGGAG GAAGCCAGCA TCCCCAAGAA GCTGCAGACC 1980
GCCAGGCCTT CAGACAGCCA GAGCAAGCGA TTGCATTCGG TCAGAACCAG CAGCAGTGGC 2040
ACCGCCCAGA GGTCCCTTTT ATCTCCACTG CATCGAAGTG GGAGCGTGGA GGACAATTAC 2100
CACACCAGCT TCCTTTTCGG CCTTTCCACC AGCCAGCAGC ACCTCACGAA GTCTGCTGGC 2160
CTGGGCCTTA AGGGCTGGCA CTCGGATATC TTGGCCCCCC AGACCTCTAC CCCTTCCCTG 2220
ACCAGCAGCT GGTATTTTGC CACAGAGTCC TCACACTTCT ACTCTGCCTC AGCCATCTAC 2280
GGAGGCAGTG CCAGTTACTC TGCCTACAGC TGCAGCCAGC TGCCCACTTG CGGAGACCAA 2340
GTCTATTCTG TGCGCAGGCG GCAGAAGCCA AGTGACAGAG CTGACTCGCG GCGGAGCTGG 2400
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Fig. 1A

SUBSTITUTE SHEET (RULE 26)

CATGAAGAGA GCCCCTTTGA AAAGCAGTTT AAACGCAGAA GCTGCCAAAT GGAATTTGGA 2460
GAGAGCATCA TGTCAGAGAA CAGGTCACGG GAAGAGCTGG GGAAAGTGGG CAGTCAGTCT 2520
AGCTTTTCGG GCAGCATGGA AATCATTGAG GTCTCCTGAG AAGAAAGACA CTTGTGACTT 2580
CTATAGACAA TTTTTTTTTC TTGTTCACAA AAAAATTCCC TGTAAATCTG AAATATATAT 2640
ATGTACATAC ATATATATTT TTGGAAAATG GAGCTATGGT GTAAAAGCAA CAGGTGGATC 2700
AACCCAGTTG TTACTCTCTT AACATCTGCA TTTGAGAGAT CAGCTAATAC TTCTCTCAAC 2760
AAAAATGGAA GGGCAGATGC TAGAATCCCC CCTAGACGGA GGAAAACCAT TTTATTCAGT 2820
GAATTACACA TCCTCTTGTT CTTAAAAAAG CAAGTGTCTT TGGTGTTGGA GGACAAAATC 2880
CCCTACCATT TTCCACGTTG TGCTACTAAG AGATCTCAAA TATTAGTCTT TGTCCGGACC 2940
CTTCCATAGT ACACCTTAGC GCTGAGACTG AGCCAGCTTG GGGGTCAGGT AGGTAGACCC 3000
TGTTAGGGAC AGAGCCTAGT GGTAAATCCA AGAGAAATGA TCCTATCCAA AGCTGATTCA 3060
CAAACCCACG CTCACCTGAC AGCCGAGGGA CACGAGCATC ACTCTGCTGG ACGGACCATT 3120
AGGGGCCTTG CCAAGGTCTA CCTTAGAGCA AACCCAGTAC CTCAGACAGG AAAGTCGGGG 3180
CTTTGACCAC TACCATATCT GGTAGCCCAT TTTCTAGGCA TTGTGAATAG GTAGGTAGCT 3240
AGTCACACTT TTCAGACCAA TTCAAACTGT CTATGCACAA AATTCCCGTG GGCCTAGATG 3300
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TGAACCACCA GGAACCTGGC AACATCACGA TTTAAGCTAA GGTTGGGAGG CTAACGAGTC 3420
TACCTCCCTC TTTGTAAATC AAAGAATTGT TTAAAATGGG ATTGTCAATC CTTTAAATAA 3480
AGATGAACTT GGTTTC   3496

Fig. 1B

MAHEMIGTQIVTERLVALLESGTEKVLLIDSRPFVEYNTSHILEAININCSKLMKRRLQQDKVLITELIQHSAKHKV
DIDCSQKVVVYDQSSQDVASLSSDCFLTVLLGKLEKSFNSVHLLAGGFAEFSRCFPGLCEGKSTLVPTCISQPCLPV
ANIGPTRILPNLYLGCQRDVLNKELMQQNGIGYVLNASNTCPKPDFIPESHFLRVPVNDSFCEKILPWLDKSVDFIE
KAKASNGCVLVHCLAGISRSATIAIAYIMKRMDMSLDEAYRFVKEKRPTISPNFNFLGQLLDYEKKIKNQTGASGPK
SKLKLLHLEKPNEPVPAVSEGGQKSETPLSPPCADSATSEAAGQRPVHPASVPSVPSVQPSLLEDSPLVQALSGLHL
SADRLEDSNKLKRSFSLDIKSVSYSASMAASLHGFSSSEDALEYYKPSTTLDGTNKLCQFSPVQELSEQTPETSPDK
EEASIPKKLQTARPSDSQSKRLHSVRTSSSGTAQRSLLSPLHRSGSVEDNYHTSFLFGLSTSQQHLTKSAGLGLKGW
HSDILAPQTSTPSLTSSWYFATESSHFYSASAIYGGSASYSAYSCSQLPTCGDQVYSVRRRQKPSDRADSRRSWHEE
SPFEKQFKRRSCQMEFGESIMSENRSREELGKVGSQSSFSGSMEIIEVS

Fig. 2

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GAGAGAAGGA GAAGATAATA TACTGAAAAG AAGAGGAGGA GGAGAGCGAC GGGACGGGAC 60
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GAGGGGGGCC CGAGGGGAGA CGCCGTGACA ACTTTCGTTT CCCTCTGAGG GAATTGGGAG 180
GTCGGCGGCC CCAAAAGCTT TCAGTCCAGT GTAAAGCTGT TGGAGCGCGG GAGCAAAGGT 240
AAAGAATGAT GTAATGCGCT GGCTGCTCCA AAGCATCTTT TGTTGTGGAA TGGTTATTCC 300
AGTCATCTCT TTATGAATCA AATGTGAGGG GCTGCTTTGT GGACGGAGTC CTTTGCAAGA 360
GCACATCAAC GGGAAAGAGA AAGAGACATT CACTTGGAGG GCTCTTGCTG AAAATGGGTT 420
TAACTCTCCT TTTGCCAGTC ACCACCAGCC TGACCTCATA CACTTTTAGT ACAATGGAGT 480
GGCTGAGCCT TTGAGCACAC CACCATTACA TCATCGTGGC AAATTAAAGA AGGAGGTGGG 540
AAAAGAGGAC TTATTGTTGT CATGGCCCAT GAGATGATTG GAACTCAAAT TGTTACTGAG 600
AGGTTGGTGG CTCTGCTGGA AAGTGGAACG GAAAAAGTGC TGCTAATTGA TAGCCGGCCA 660
TTTGTGGAAT ACAATACATC CCACATTTTG GAAGCCATTA ATATCAACTG CTCCAAGCTT 720
ATGAAGCGAA GGTTGCAACA GGACAAAGTG TTAATTACAG AGCTCATCCA GCATTCAGCG 780
AAACATAAGG TTGACATTGA TTGCAGTCAG AAGGTTGTAG TTTACGATCA AAGCTCCCAA 840
GATGTTGCCT CTCTCTCTTC AGACTGTTTT CTCACTGTAC TTCTGGGTAA ACTGGAGAAG 900
AGCTTCAACT CTGTTCACCT GCTTGCAGGA GCTGATGCAG CAGAATGGGA TTGGTTATGT 960
GTTAAATGCC AGCAATACCT GTCCAAAGCC TGACTTTATC CCCGAGTCTC ATTTCCTGCG 1020
TGTGCCTGTG AATGACAGCT TTTGTGAGAA AATTTTGCCG TGGTTGGACA AATCAGTAGA 1080
TTTCATTGAG AAAGCAAAAG CCTCCAATGG ATGTGTTCTA GTGCACTGTT TAGCTGGGAT 1140
CTCCCGCTCC GCCACCATCG CTATCGCCTA CATCATGAAG AGGATGGACA TGTCTTTAGA 1200
TGAAGCTTAC AGATTTGTGA AAGAAAAAAG ACCTACTATA TCTCCAAACT TCAATTTTCT 1260
GGGCCAACTC CTGGACTATG AGAAGAAGAT TAAGAACCAG ACTGGAGCAT CAGGGCCAAA 1320
GAGCAAACTC AAGCTGCTGC ACCTGGAGAA GCCAAATGAA CCTGTCCCTG CTGTCTCAGA 1380
GGGTGGACAG AAAAGCGAGA CGCCCCTCAG TCCACCCTGT GCCGACTCTG CTACCTCAGA 1440
GGCAGCAGGA CAAAGGCCCG TGCATCCCGC CAGCGTGCCC AGCGTGCCCA GCGTGCAGCC 1500
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ATACTACAAA CCTTCCACTA CTCTGGATGG GACCAACAAG CTATGCCAGT TCTCCCCTGT 1740
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CAAGAAGCTG CAGACCGCCA GGCCTTCAGA CAGCCAGAGC AAGCGATTGC ATTCGGTCAG 1860
AACCAGCAGC AGTGGCACCG CCCAGAGGTC CCTTTTATCT CCACTGCATC GAAGTGGGAG 1920
CGTGGAGGAC AATTACCACA CCAGCTTCCT TTTCGGCCTT TCCACCAGCC AGCAGCACCT 1980
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CACTTGCGGA GACCAAGTCT ATTCTGTGCG CAGGCGGCAG AAGCCAAGTG ACAGAGCTGA 2220
CTCGCGGCGG AGCTGGCATG AAGAGAGCCC CTTTGAAAAG CAGTTTAAAC GCAGAAGCTG 2280
CCAAATGGAA TTTGGAGAGA GCATCATGTC AGAGAACAGG TCACGGGAAG AGCTGGGGAA 2340
AGTGGGCAGT CAGTCTAGCT TTTCGGGCAG CATGGAAATC ATTGAGGTCT CCTGAGAAGA 2400
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Fig. 3A

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AAGACACTTG TGACTTCTAT AGACAATTTT TTTTTCTTGT TCACAAAAAA ATTCCCTGTA 2460
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AAGCAACAGG TGGATCAACC CAGTTGTTAC TCTCTTAACA TCTGCATTTG AGAGATCAGC 2580
TAATACTTCT CTCAACAAAA ATGGAAGGGC AGATGCTAGA ATCCCCCCTA GACGGAGGAA 2640
AACCATTTTA TTCAGTGAAT TACACATCCT CTTGTTCTTA AAAAAGCAAG TGTCTTTGGT 2700
GTTGGAGGAC AAAATCCCCT ACCATTTTCC ACGTTGTGCT ACTAAGAGAT CTCAAATATT 2760
AGTCTTTGTC CGGACCCTTC CATAGTACAC CTTAGCGCTG AGACTGAGCC AGCTTGGGGG 2820
TCAGGTAGGT AGACCCTGTT AGGGACAGAG CCTAGTGGTA AATCCAAGAG AAATGATCCT 2880
ATCCAAAGCT GATTCACAAA CCCACGCTCA CCTGACAGCC GAGGGACACG AGCATCACTC 2940
TGCTGGACGG ACCATTAGGG GCCTTGCCAA GGTCTACCTT AGAGCAAACC CAGTACCTCA 3000
GACAGGAAAG TCGGGGCTTT GACCACTACC ATATCTGGTA GCCCATTTTC TAGGCATTGT 3060
GAATAGGTAG GTAGCTAGTC ACACTTTTCA GACCAATTCA AACTGTCTAT GCACAAAATT 3120
CCCGTGGGCC TAGATGGAGA TAATTTTTTT TTCTTCTCAG CTTTATGAAG AGAAGGGAAA 3180
CTGTCTAGGA TTCAGCTGAA CCACCAGGAA CCTGGCAACA TCACGATTTA AGCTAAGGTT 3240
GGGAGGCTAA CGAGTCTACC TCCCTCTTTG TAAATCAAAG AATTGTTTAA AATGGGATTG 3300
TCAATCCTTT AAATAAAGAT GAACTTGGTT TC                              3332
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Fig. 3B

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MLPLSLQTVFSLYFWVNWRRASTLFTCLQELMQQNGIGYVLNASNTCPKPDFIPESHFLRVPVNDSFCEKILPWLDK
SVDFIEKAKASNGCVLVHCLAGISRSATIAIAYIMKRMDMSLDEAYRFVKEKRPTISPNFNFLGQLLDYEKKIKNQT
GASGPKSKLKLLHLEKPNEPVPAVSEGGQKSETPLSPPCADSATSEAAGQRPVHPASVPSVQPSLLEDSPLVQA
LSGLHLSADRLEDSNKLKRSFSLDIKSVSYSASMAASLHGFSSSEDALEYYKPSTTLDGTNKLCQFSPVQELSEQTP
ETSPDKEEASIPKKLQTARPSDSQSKRLHSVRTSSSGTAQRSLLSPLHRSGSVEDNYHTSFLFGLSTSQQHLTKSAG
LGLKGWHSDILAPQTSTPSLTSSWYFATESSHFYSASAIYGGSASYSAYSCSQLPTCGDQVYSVRRRQKPSDRADSR
RSWHEESPFEKQFKRRSCQMEFGESIMSENRSREELGKVGSQSSFSGSMEIIEVS
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Fig. 4

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           1.......10........20........30........40........50........60........70........80
PYST1      DGSPLSNSQPSFPVEILPFLYLGCAKDSTNLDVLEEFGIKYILNVTP-----NLPNLFENAGEFKYKQIPISDHWSQN
MKP-7      DGSPVPSSQPAFPVQILPYLYLGCAKDSTNLDVLGKYGIKYILNVTP-----NLPNAFEHGGEFTYKQIPISDHWSQN
MKP-4      ATPPPVGLRASFPVQILPNLYLGSARDSANLESLAKLGIRYILNVTP-----NLPNFFEKNGDFHYKQIPISDHWSQN
hVH5       LSQPCLPVPSVGLTRILPHLYLGSQKDVLNKDLMTQNGISYVLNASN-------SCPKP-DFICESRFMRVPINDNYCEK
DSP-16     ISQPCLPVANIGPTRILPNLYLGCQRDVLNKELMQQNGIGYVLNASN-------TCPKP-DFIPESHFLRVPVNDSFCEK
PAC1       SDPRVPIYDQGGPVEILPYLYLGSCNHSSDLQGLQACGITAVLNVSA------SCPNHFE--GLFHYKSIPVEDNQMVE
MKP-1      SSCSTPLYDQGGPVEILPFLYLGSAYHASRKDMLDALGITALINVSA------NCPNHFE--GHYQYKSIPVEDNHKAD
MKP-2      SSCGTPLHDQGGPVEILPFLYLGSAYHAARRDMLDALGITALLNVSS------DCPNHFE--GHYQYKCIPVEDNHKAD
MKP-5      NVSYRPAYDQGGPVEILPFLYLGSAYHASKCEFLANLHITALLNVSR------RTSEACM--THLHYKWIPVEDSHTAD
VHR        DGSGCYSLPSQPCNEVTPRIYVGNASVAQDIPKLQKLGITHVLNAAEGRSFMHVNTNANFYKDSGITYLGIKANDTQEFN
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Fig. 5

1

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Pro Phe Val Glu Tyr Asn Thr Ser His Ile Leu Glu Ala Ile Asn Ile
            35                  40                  45
Asn Cys Ser Lys Leu Met Lys Arg Arg Leu Gln Gln Asp Lys Val Leu
    50                  55                  60
Ile Thr Glu Leu Ile Gln His Ser Ala Lys His Lys Val Asp Ile Asp
65                  70                  75                  80
Cys Ser Gln Lys Val Val Val Tyr Asp Gln Ser Ser Gln Asp Val Ala
                85                  90                  95
Ser Leu Ser Ser Asp Cys Phe Leu Thr Val Leu Leu Gly Lys Leu Glu
            100                 105                 110
Lys Ser Phe Asn Ser Val His Leu Leu Ala Gly Gly Phe Ala Glu Phe
            115                 120                 125
Ser Arg Cys Phe Pro Gly Leu Cys Glu Gly Lys Ser Thr Leu Val Pro
    130                 135                 140
Thr Cys Ile Ser Gln Pro Cys Leu Pro Val Ala Asn Ile Gly Pro Thr
145                 150                 155                 160
Arg Ile Leu Pro Asn Leu Tyr Leu Gly Cys Gln Arg Asp Val Leu Asn
                165                 170                 175
Lys Glu Leu Met Gln Gln Asn Gly Ile Gly Tyr Val Leu Asn Ala Ser
            180                 185                 190
Asn Thr Cys Pro Lys Pro Asp Phe Ile Pro Glu Ser His Phe Leu Arg
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Lys Ser Val Asp Phe Ile Glu Lys Ala Lys Ala Ser Asn Gly Cys Val
225                    230                    235                    240
Leu Val His Cys Leu Ala Gly Ile Ser Arg Ser Ala Thr Ile Ala Ile
                  245                    250                    255
Ala Tyr Ile Met Lys Arg Met Asp Met Ser Leu Asp Glu Ala Tyr Arg
                  260                    265                    270
Phe Val Lys Glu Lys Arg Pro Thr Ile Ser Pro Asn Phe Asn Phe Leu
              275                    280                    285
Gly Gln Leu Leu Asp Tyr Glu Lys Lys Ile Lys Asn Gln Thr Gly Ala
         290                    295                    300
Ser Gly Pro Lys Ser Lys Leu Lys Leu Leu His Leu Glu Lys Pro Asn
305                    310                    315                    320
Glu Pro Val Pro Ala Val Ser Glu Gly Gly Gln Lys Ser Glu Thr Pro
                  325                    330                    335
Leu Ser Pro Pro Cys Ala Asp Ser Ala Thr Ser Glu Ala Ala Gly Gln
              340                    345                    350
Arg Pro Val His Pro Ala Ser Val Pro Ser Val Pro Ser Val Gln Pro
         355                    360                    365
Ser Leu Leu Glu Asp Ser Pro Leu Val Gln Ala Leu Ser Gly Leu His
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Leu Ser Ala Asp Arg Leu Glu Asp Ser Asn Lys Leu Lys Arg Ser Phe
385                    390                    395                    400
Ser Leu Asp Ile Lys Ser Val Ser Tyr Ser Ala Ser Met Ala Ala Ser
                  405                    410                    415
Leu His Gly Phe Ser Ser Ser Glu Asp Ala Leu Glu Tyr Tyr Lys Pro
              420                    425                    430
Ser Thr Thr Leu Asp Gly Thr Asn Lys Leu Cys Gln Phe Ser Pro Val
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Gln Glu Leu Ser Glu Gln Thr Pro Glu Thr Ser Pro Asp Lys Glu Glu
    450                    455                    460
Ala Ser Ile Pro Lys Lys Leu Gln Thr Ala Arg Pro Ser Asp Ser Gln
465                    470                    475                    480
Ser Lys Arg Leu His Ser Val Arg Thr Ser Ser Ser Gly Thr Ala Gln
              485                    490                    495
Arg Ser Leu Leu Ser Pro Leu His Arg Ser Gly Ser Val Glu Asp Asn
              500                    505                    510
Tyr His Thr Ser Phe Leu Phe Gly Leu Ser Thr Ser Gln Gln His Leu
         515                    520                    525
Thr Lys Ser Ala Gly Leu Gly Leu Lys Gly Trp His Ser Asp Ile Leu
         530                    535                    540
Ala Pro Gln Thr Ser Thr Pro Ser Leu Thr Ser Ser Trp Tyr Phe Ala
545                    550                    555                    560
Thr Glu Ser Ser His Phe Tyr Ser Ala Ser Ala Ile Tyr Gly Gly Ser
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Ala Ser Tyr Ser Ala Tyr Ser Cys Ser Gln Leu Pro Thr Cys Gly Asp
         580                    585                    590
Gln Val Tyr Ser Val Arg Arg Arg Gln Lys Pro Ser Asp Arg Ala Asp
    595                    600                    605
Ser Arg Arg Ser Trp His Glu Glu Ser Pro Phe Glu Lys Gln Phe Lys
    610                    615                    620
Arg Arg Ser Cys Gln Met Glu Phe Gly Glu Ser Ile Met Ser Glu Asn
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Val Leu Glu Glu Phe Gly Ile Lys Tyr Ile Leu Asn Val Thr Pro Asn
        35   40   45
Leu Pro Asn Leu Phe Glu Asn Ala Gly Glu Phe Lys Tyr Lys Gln Ile
    50   55   60
Pro Ile Ser Asp His Trp Ser Gln Asn Leu Ser Gln Phe Phe Pro Glu
65   70   75   80
Ala Ile Ser Phe Ile Asp Glu Ala Arg Gly Lys Asn Cys Gly Val Leu
                85   90   95
Val His Cys Leu Ala Gly Ile Ser Arg Ser Val Thr Val Thr Val Ala
            100   105   110
Tyr Leu Met Gln Lys Leu Asn Leu Ser Met Asn Asp Ala Tyr Asp Ile
            115   120   125
Val Lys Met Lys Lys Ser Asn Ile Ser Pro Asn Phe Asn Phe Met Gly
        130   135   140
Gln Leu Leu Asp Phe Glu Arg Thr Leu Gly Leu Ser
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Val Leu Gly Lys Tyr Gly Ile Lys Tyr Ile Leu Asn Val Thr Pro Asn
        35   40   45
Leu Pro Asn Ala Phe Glu His Gly Gly Glu Phe Thr Tyr Lys Gln Ile
    50   55   60
Pro Ile Ser Asp His Trp Ser Gln Asn Leu Ser Gln Phe Phe Pro Glu
65   70   75   80
Ala Ile Ser Phe Ile Asp Glu Ala Arg Ser Lys Lys Cys Gly Val Leu
                85   90   95
Val His Cys Leu Ala Gly Ile Ser Arg Ser Val Thr Val Thr Val Ala
            100   105   110
Tyr Leu Met Gln Lys Met Asn Leu Ser Leu Asn Asp Ala Tyr Asp Phe
            115   120   125
Val Lys Arg Lys Lys Ser Asn Ile Ser Pro Asn Phe Asn Phe Met Gly
        130   135   140
Gln Leu Leu Asp Phe Glu Arg Thr Leu Gly Leu Ser
145   150   155

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Ser Leu Ala Lys Leu Gly Ile Arg Tyr Ile Leu Asn Val Thr Pro Asn
        35   40   45
Leu Pro Asn Phe Phe Glu Lys Asn Gly Asp Phe His Tyr Lys Gln Ile
        50   55   60
Pro Ile Ser Asp His Trp Ser Gln Asn Leu Ser Arg Phe Phe Pro Glu
65   70   75   80
Ala Ile Glu Phe Ile Asp Glu Ala Leu Ser Gln Asn Cys Gly Val Leu
                85   90   95
Val His Cys Leu Ala Gly Val Ser Arg Ser Val Thr Val Thr Val Ala
            100   105   110
Tyr Leu Met Gln Lys Leu His Leu Ser Leu Asn Asp Ala Tyr Asp Leu
        115   120   125
Val Lys Arg Lys Lys Ser Asn Ile Ser Pro Asn Phe Asn Phe Met Gly
    130   135   140
Gln Leu Leu Asp Phe Glu Arg Ser Leu Arg Leu Glu
145   150   155

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            20   25   30
Leu Met Thr Gln Asn Gly Ile Ser Tyr Val Leu Asn Ala Ser Asn Ser
        35   40   45
Cys Pro Lys Pro Asp Phe Ile Cys Glu Ser Arg Phe Met Arg Val Pro
        50   55   60
Ile Asn Asp Asn Tyr Cys Glu Lys Leu Leu Pro Trp Leu Asp Lys Ser
65   70   75   80
Ile Glu Phe Ile Asp Lys Ala Lys Leu Ser Ser Cys Gln Val Ile Val
                85   90   95
His Cys Leu Ala Gly Ile Ser Arg Ser Ala Thr Ile Ala Ile Ala Tyr
            100   105   110
Ile Met Lys Thr Met Gly Met Ser Ser Asp Asp Ala Tyr Arg Phe Val
        115   120   125
Lys Asp Arg Arg Pro Ser Ile Ser Pro Asn Phe Asn Phe Leu Gly Gln
    130   135   140
Leu Leu Glu Tyr Glu Arg Thr Leu Lys Leu Leu
145   150   155

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Leu Met Gln Gln Asn Gly Ile Gly Tyr Val Leu Asn Ala Ser Asn Thr
        35   40   45
Cys Pro Lys Pro Asp Phe Ile Pro Glu Ser His Phe Leu Arg Val Pro
    50   55   60
Val Asn Asp Ser Phe Cys Glu Lys Ile Leu Pro Trp Leu Asp Lys Ser
65   70   75   80
Val Asp Phe Ile Glu Lys Ala Lys Ala Ser Asn Gly Cys Val Leu Val
            85   90   95
His Cys Leu Ala Gly Ile Ser Arg Ser Ala Thr Ile Ala Ile Ala Tyr
            100   105   110
Ile Met Lys Arg Met Asp Met Ser Leu Asp Glu Ala Tyr Arg Phe Val
        115   120   125
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Leu Leu Asp Tyr Glu Lys Lys Ile Lys Asn Gln
145   150   155

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Gly Leu Gln Ala Cys Gly Ile Thr Ala Val Leu Asn Val Ser Ala Ser
        35   40   45
Cys Pro Asn His Phe Glu Gly Leu Phe His Tyr Lys Ser Ile Pro Val
    50   55   60
Glu Asp Asn Gln Met Val Glu Ile Ser Ala Trp Phe Gln Glu Ala Ile
65   70   75   80
Ser Phe Ile Asp Ser Val Lys Asn Ser Gly Gly Arg Val Leu Val His
            85   90   95
Cys Gln Ala Gly Ile Ser Arg Ser Ala Thr Ile Cys Leu Ala Tyr Leu
            100   105   110
Ile Gln Ser His Arg Val Arg Leu Asp Glu Ala Phe Asp Phe Val Lys
        115   120   125
Gln Arg Arg Gly Val Ile Ser Pro Asn Phe Ser Phe Met Gly Gln Leu
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Leu Gln Leu Glu Thr Gln Val Leu Cys His
145   150

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Met Leu Asp Ala Leu Gly Ile Thr Ala Leu Ile Asn Val Ser Ala Asn
            35   40   45
Cys Pro Asn His Phe Glu Gly His Tyr Gln Tyr Lys Ser Ile Pro Val
    50   55   60
Glu Asp Asn His Lys Ala Asp Ile Ser Ser Trp Phe Asn Glu Ala Ile
65   70   75   80
Asp Phe Ile Asp Ser Ile Lys Asn Ala Gly Gly Arg Val Phe Val His
            85   90   95
Cys Gln Ala Gly Ile Ser Arg Ser Ala Thr Ile Cys Leu Ala Tyr Leu
            100   105   110
Met Arg Thr Asn Arg Val Lys Leu Asp Glu Ala Phe Glu Phe Val Lys
            115   120   125
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Met Leu Asp Ala Leu Gly Ile Thr Ala Leu Leu Asn Val Ser Ser Asp
            35   40   45
Cys Pro Asn His Phe Glu Gly His Tyr Gln Tyr Lys Cys Ile Pro Val
    50   55   60
Glu Asp Asn His Lys Ala Asp Ile Ser Ser Trp Phe Met Glu Ala Ile
65   70   75   80
Glu Tyr Ile Asp Ala Val Lys Asp Cys Arg Gly Arg Val Leu Val His
            85   90   95
Cys Gln Ala Gly Ile Ser Arg Ser Ala Thr Ile Cys Leu Ala Tyr Leu
            100   105   110
Met Met Lys Lys Arg Val Arg Leu Glu Glu Ala Phe Glu Phe Val Lys
            115   120   125
Gln Arg Arg Ser Ile Ile Ser Pro Asn Phe Ser Phe Met Gly Gln Leu
        130   135   140
Leu Gln Phe Glu Ser Gln Val Leu Ala Thr
145   150

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Phe Leu Ala Asn Leu His Ile Thr Ala Leu Leu Asn Val Ser Arg Arg
        35   40   45
Thr Ser Glu Ala Cys Met Thr His Leu His Tyr Lys Trp Ile Pro Val
        50   55   60
Glu Asp Ser His Thr Ala Asp Ile Ser Ser His Phe Gln Glu Ala Ile
65   70   75   80
Asp Phe Ile Asp Cys Val Arg Glu Lys Gly Gly Lys Val Leu Val His
                85   90   95
Cys Glu Ala Gly Ile Ser Arg Ser Pro Thr Ile Cys Met Ala Tyr Leu
                100   105   110
Met Lys Thr Lys Gln Phe Arg Leu Lys Glu Ala Phe Asp Tyr Ile Lys
            115   120   125
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Leu Gln Tyr Glu Ser Glu Ile Leu Pro Ser
145   150

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        35   40   45
Arg Ser Phe Met His Val Asn Thr Asn Ala Asn Phe Tyr Lys Asp Ser
        50   55   60
Gly Ile Thr Tyr Leu Gly Ile Lys Ala Asn Asp Thr Gln Glu Phe Asn
65   70   75   80
Leu Ser Ala Tyr Phe Glu Arg Ala Ala Asp Phe Ile Asp Gln Ala Leu
                85   90   95
Ala Gln Lys Asn Gly Arg Val Leu Val His Cys Arg Glu Gly Tyr Ser
            100   105   110
Arg Ser Pro Thr Leu Val Ile Ala Tyr Leu Met Met Arg Gln Lys Met
        115   120   125
Asp Val Lys Ser Ala Leu Ser Tyr Val Arg Gln Asn Arg Glu Ile Gly
        130   135   140
Pro Asn Asp Gly Phe Leu Ala Gln Leu Cys Gln Leu Asn Asp Arg Leu
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Ala Lys Glu

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            20                  25                  30
Asp Met Ser Leu Asp Glu Ala Tyr Arg
        35                  40


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<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer

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<210> 15
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1               5                   10


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      amino acid sequences.

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Thr Asn Ile Leu Ala Tyr Leu Met
            20


<210> 18
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<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> RACE primer

<400> 18
catttcctgc gtgtgcctgt gaatgacagc tt                                      32


<210> 19
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> RACE primer

<400> 19
aagagagaga ggcaacatct tgggagcttt gatcg                                   35


<210> 20
<211> 3332
<212> DNA
<213> Homo sapiens

<400> 20
gagagaagga gaagataata tactgaaaag aagaggagga ggagagcgac gggacgggac  60
gcgagcggga gcgcagccgc cctctcggct ccgcggcggc gcctcgcaag tccgggaggc  120
gaggggggcc cgaggggaga cgccgtgaca actttcgttt ccctctgagg gaattgggag  180
gtcggcggcc ccaaaagctt tcagtccagt gtaaagctgt tggagcgcgg gagcaaaggt  240
aaagaatgat gtaatgcgct ggctgctcca aagcatcttt tgttgtggaa tggttattcc  300
agtcatctct ttatgaatca aatgtgaggg gctgctttgt ggacggagtc ctttgcaaga  360
gcacatcaac gggaaagaga aagagacatt cacttggagg gctcttgctg aaaatgggtt  420
taactctcct tttgccagtc accaccagcc tgacctcata cacttttagt acaatggagt  480
ggctgagcct ttgagcacac caccattaca tcatcgtggc aaattaaaga aggaggtggg  540
aaaagaggac ttattgttgt catggcccat gagatgattg gaactcaaat tgttactgag  600
aggttggtgg ctctgctgga aagtggaacg gaaaaagtgc tgctaattga tagccggcca  660
tttgtggaat acaatacatc ccacattttg gaagccatta atatcaactg ctccaagctt  720
atgaagcgaa ggttgcaaca ggacaaagtg ttaattacag agctcatcca gcattcagcg  780
aaacataagg ttgacattga ttgcagtcag aaggttgtag tttacgatca aagctcccaa  840
gatgttgcct ctctctcttc agactgtttt ctcactgtac ttctgggtaa actggagaag  900
agcttcaact ctgttcacct gcttgcagga gctgatgcag cagaatggga ttggttatgt  960
gttaaatgcc agcaatacct gtccaaagcc tgactttatc cccgagtctc atttcctgcg  1020
tgtgcctgtg aatgacagct tttgtgagaa aattttgccg tggttggaca aatcagtaga  1080
tttcattgag aaagcaaaag cctccaatgg atgtgttcta gtgcactgtt tagctgggat  1140
ctcccgctcc gccaccatcg ctatcgccta catcatgaag aggatggaca tgtctttaga  1200
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gggccaactc ctggactatg agaagaagat taagaaccag actggagcat cagggccaaa  1320
gagcaaactc aagctgctgc acctggagaa gccaaatgaa cctgtccctg ctgtctcaga  1380
gggtggacag aaaagcgaga cgcccctcag tccacccgt gccgactctg ctacctcaga  1440
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gtcgctgtta gaggacagcc cgctggtaca ggcgctcagt gggctgcacc tgtccgcaga  1560
caggctggaa gacagcaata agctcaagcg ttccttctct ctggatatca aatcagtttc  1620
atattcagcc agcatggcag catccttaca tggcttctcc tcatcagaag atgctttgga  1680
atactacaaa ccttccacta ctctggatgg gaccaacaag ctatgccagt ctctcccctgt  1740
tcaggaacta tcggagcaga ctcccgaaac cagtcctgat aaggaggaag ccagcatccc  1800
caagaagctg cagaccgcca ggccttcaga cagccagagc aagcgattgc attcggtcag  1860
```

```
aaccagcagc agtggcaccg cccagaggtc ccttttatct ccactgcatc gaagtgggag 1920
cgtggaggac aattaccaca ccagcttcct tttcggcctt tccaccagcc agcagcacct 1980
cacgaagtct gctggcctgg gccttaaggg ctggcactcg gatatcttgg cccccccagac 2040
ctctacccct tccctgacca gcagctggta ttttgccaca gagtcctcac acttctactc 2100
tgcctcagcc atctacggag gcagtgccag ttactctgcc tacagctgca gccagctgcc 2160
cacttgcgga gaccaagtct attctgtgcg caggcggcag aagccaagtg acagagctga 2220
ctcgcggcgg agctggcatg aagagagccc ctttgaaaag cagtttaaac gcagaagctg 2280
ccaaatggaa tttggagaga gcatcatgtc agagaacagg tcacgggaag agctggggaa 2340
agtgggcagt cagtctagct tttcgggcag catggaaatc attgaggtct cctgagaaga 2400
aagacacttg tgacttctat agacaatttt tttttcttgt tcacaaaaaa attccctgta 2460
aatctgaaat atatatatgt acatacatat atattttttgg aaaatggagc tatggtgtaa 2520
aagcaacagg tggatcaacc cagttgttac tctcttaaca tctgcatttg agagatcagc 2580
taatacttct ctcaacaaaa atggaagggc agatgctaga atcccccccta gacggaggaa 2640
aaccatttta ttcagtgaat tacacatcct cttgttctta aaaaagcaag tgtctttggt 2700
gttggaggac aaaatcccct accattttcc acgttgtgct actaagagat ctcaaatatt 2760
agtctttgtc cggacccttc catagtacac cttagcgctg agactgagcc agcttggggg 2820
tcaggtaggt agaccctgtt agggacagag cctagtggta aatccaagag aaatgatcct 2880
atccaaagct gattcacaaa cccacgctca cctgacagcc gagggacacg agcatcactc 2940
tgctggacgg accattaggg gccttgccaa ggtctacctt agagcaaacc cagtacctca 3000
gacaggaaag tcggggcttt gaccactacc atatctggta gcccattttc taggcattgt 3060
gaataggtag gtagctagtc acactttttca gaccaattca aactgtctat gcacaaaatt 3120
cccgtgggcc tagatggaga taattttttt ttcttctcag ctttatgaag agaagggaaa 3180
ctgtctagga ttcagctgaa ccaccaggaa cctggcaaca tcacgattta agctaaggtt 3240
gggaggctaa cgagtctacc tccctctttg taaatcaaag aattgtttaa aatgggattg 3300
tcaatccttt aaataaagat gaacttggtt tc                                 3332
```

<210> 21
<211> 517
<212> PRT
<213> Homo sapiens

<400> 21
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Met Leu Pro Leu Ser Leu Gln Thr Val Phe Ser Leu Tyr Phe Trp Val
1               5                  10                  15
Asn Trp Arg Arg Ala Ser Thr Leu Phe Thr Cys Leu Gln Glu Leu Met
            20                  25                  30
Gln Gln Asn Gly Ile Gly Tyr Val Leu Asn Ala Ser Asn Thr Cys Pro
        35                  40                  45
Lys Pro Asp Phe Ile Pro Glu Ser His Phe Leu Arg Val Pro Val Asn
    50                  55                  60
Asp Ser Phe Cys Glu Lys Ile Leu Pro Trp Leu Asp Lys Ser Val Asp
65                  70                  75                  80
Phe Ile Glu Lys Ala Lys Ala Ser Asn Gly Cys Val Leu Val His Cys
                85                  90                  95
Leu Ala Gly Ile Ser Arg Ser Ala Thr Ile Ala Ile Ala Tyr Ile Met
            100                 105                 110
Lys Arg Met Asp Met Ser Leu Asp Glu Ala Tyr Arg Phe Val Lys Glu
        115                 120                 125
Lys Arg Pro Thr Ile Ser Pro Asn Phe Asn Phe Leu Gly Gln Leu Leu
    130                 135                 140
Asp Tyr Glu Lys Lys Ile Lys Asn Gln Thr Gly Ala Ser Gly Pro Lys
145                 150                 155                 160
Ser Lys Leu Lys Leu Leu His Leu Glu Lys Pro Asn Glu Pro Val Pro
                165                 170                 175
Ala Val Ser Glu Gly Gly Gln Lys Ser Glu Thr Pro Leu Ser Pro Pro
            180                 185                 190
Cys Ala Asp Ser Ala Thr Ser Glu Ala Ala Gly Gln Arg Pro Val His
        195                 200                 205
```

```
Pro Ala Ser Val Pro Ser Val Pro Ser Val Gln Pro Ser Leu Leu Glu
    210             215             220
Asp Ser Pro Leu Val Gln Ala Leu Ser Gly Leu His Leu Ser Ala Asp
225             230             235             240
Arg Leu Glu Asp Ser Asn Lys Leu Lys Arg Ser Phe Ser Leu Asp Ile
            245             250             255
Lys Ser Val Ser Tyr Ser Ala Ser Met Ala Ala Ser Leu His Gly Phe
            260             265             270
Ser Ser Ser Glu Asp Ala Leu Glu Tyr Tyr Lys Pro Ser Thr Thr Leu
        275             280             285
Asp Gly Thr Asn Lys Leu Cys Gln Phe Ser Pro Val Gln Glu Leu Ser
    290             295             300
Glu Gln Thr Pro Glu Thr Ser Pro Asp Lys Glu Glu Ala Ser Ile Pro
305             310             315             320
Lys Lys Leu Gln Thr Ala Arg Pro Ser Asp Ser Gln Ser Lys Arg Leu
            325             330             335
His Ser Val Arg Thr Ser Ser Ser Gly Thr Ala Gln Arg Ser Leu Leu
            340             345             350
Ser Pro Leu His Arg Ser Gly Ser Val Glu Asp Asn Tyr His Thr Ser
        355             360             365
Phe Leu Phe Gly Leu Ser Thr Ser Gln Gln His Leu Thr Lys Ser Ala
    370             375             380
Gly Leu Gly Leu Lys Gly Trp His Ser Asp Ile Leu Ala Pro Gln Thr
385             390             395             400
Ser Thr Pro Ser Leu Thr Ser Ser Trp Tyr Phe Ala Thr Glu Ser Ser
            405             410             415
His Phe Tyr Ser Ala Ser Ala Ile Tyr Gly Gly Ser Ala Ser Tyr Ser
            420             425             430
Ala Tyr Ser Cys Ser Gln Leu Pro Thr Cys Gly Asp Gln Val Tyr Ser
        435             440             445
Val Arg Arg Arg Gln Lys Pro Ser Asp Arg Ala Asp Ser Arg Arg Ser
    450             455             460
Trp His Glu Glu Ser Pro Phe Glu Lys Gln Phe Lys Arg Arg Ser Cys
465             470             475             480
Gln Met Glu Phe Gly Glu Ser Ile Met Ser Glu Asn Arg Ser Arg Glu
            485             490             495
Glu Leu Gly Lys Val Gly Ser Gln Ser Ser Phe Ser Gly Ser Met Glu
        500             505             510
Ile Ile Glu Val Ser
        515
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<210> 22
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Autophosphorylation site from EGF receptor.

<400> 22
Asp Ala Asp Glu Tyr Leu
 1               5
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